Storage Stability and Improvement of

Intermediate Moisture Foods

CONTRACT NAS 9-12560
PHASE III

Final Report
August 1974 to August 1975

National Aeronautics and Space Administration
Food and Nutrition Office
Houston, Texas   77058

Principal Investigator
Dr. Theodore P. Labuza
Professor of Food Technology
Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

ABSTRACT

This study was designed to determine methods for the improvement

of shelf-life stability of intermediate moisture foods (IMF). Microbial

challenge studies showed that protection against molds and Staphylococcus

aureus could be achieved by a combination of antimicrobial agents,

humectants and food acids. Potassium sorbate and propylene glycol

gave the best results. It was also confirmed that the maximum in heat

resistance shown by vegetative pathogens at intermediate water activities

also occurred in a solid food. Non-enzymatic browning is a complex

reaction and protein substitution without proper shelf-life testing

can result in product unacceptability. Glycols and sorbitol both

achieve browning inhibition due to their action as a medium for reaction

and effect on viscosity of the adsorbed phase. Chemical availability

results showed rapid lysine loss before visual discoloration occurred.

This is being confirmed with a biological test using Tetrahymena

pyriformis W. Accelerated temperature tests show that one can quickly

predict the effectiveness of food antioxidants against rancidity

development, however, the protection factor changes with temperature.

BHA was found to be the best antioxidant for iron catalyzed oxidation.

SUMMARY AND RECOMMENDATIONS

This study was a continuation of Contract NAS 9-12560 which was designed to determine methods for improvement of shelf-life stability of intermediate moisture foods (IMF). Areas under investigation were microbiological growth in IMF, microbiological death during processing, effects of food components on non-enzymatic browning, development of a rapid biological lysine loss test method, investigation of food humectant water binding properties, measurement of water activity (a_w) and accelerated shelf-life testing of antioxidants. Based on the results obtained, several recommendations can be made for either shelf-life improvement or for further work. These were as follows:

1. With respect to microbial inhibition:

   a. Most mold inhibitors by themselves do not inhibit Staphylococcus aureus growth at a_w 0.86 to 0.90 if the pH is >5.6.

   b. Acidification to pH 5.2 improves the effectiveness of both mold and S. aureus inhibitors.

   c. At pH 5.2 many common mold inhibitors also inhibit staphylococcal growth.

   d. Propylene glycol at 4-6% in combination with potassium sorbate or calcium propionate at 0.1 to 0.3% are totally effective inhibitors at pH 5 to 6.

2. With respect to microbial death and water activity:

   a. The maximum heat resistance for the pathogenic species of Staphylococcus sp. and Salmonellae sp. is in the range of

0.80 to 0.85. The heat resistance is 10 to 100 times as great as at a_w 1.0.

    b. Based on this, potential problem food ingredients should be prepasteurized before combination and processing at IMF water activities.

3. With respect to non-enzymatic browning:

    a. If, due to various reasons, a new protein source or type is substituted for another in an IMF product a rapid shelf-life test should be run to determine if browning or lysine loss occur more rapidly than with the original protein.

    b. Liquid glycols such as glycerol and propylene glycol inhibit the browning reaction at high a_w when added at 20%. The effect at lower levels needs to be investigated.

    c. Sorbitol also inhibits browning due to its viscosity.

    d. Browning has a high Q_{10} of 5 to 6 thus high temperatures in processing and storage should be avoided.

    e. Model system results show rapid lysine loss before darkening of color occurs. This needs to be confirmed in biological tests.

    f. Tetrahymena pyriformis W seems applicable for rapid testing of lysine degradation in browning.

4. With respect to humectants:

    a. Of the glycols, propylene glycol would be the best humectant to use because of its high water binding, its action as a microbial inhibitor and its action as an inhibitor of non-enzymatic browning.

b. Sorbitol has the best water binding properties of the sugars and acts to inhibit browning.

c. Sodium and potassium chloride are the best humectants but are limited in use because of flavor.

5. With respect to water activity measurement:

a. There is a lack of standards for comparison of values between laboratories thus these must be developed.

b. Water activity should only be reported to two decimal places.

c. The vapor pressure manometer is the best overall technique for a_w measurement.

6. With respect to antioxidant testing:

a. The effectiveness of various antioxidants is not the same at both dry and intermediate moisture content. For example, propyl gallate which is effective in dry systems acts as a pro-oxidant in the IM range.

b. Antioxidant effectiveness decreases with increasing temperature, which makes it difficult to project IM food shelf life from accelerated tests.

c. BHA and BHT are strong antioxidants for Fe (II) catalyzed lipid oxidation and give an increase in shelf-life of 10 to 40 times.

d. THHQ is not an effective antioxidant.

7. With respect to cheese and water activity:

a. Most processed cheeses have a high (>0.94) water activity. Their stability is thus subject to pasteurization and good packaging.

b. An acceptable IM cheese of a_w 0.82 and pH 5.2 could be made by adding propylene glycol, non-fat dry milk solids and salt. The cheese should be stable because of the effective anti-mycotic system used.

TABLE OF CONTENTS

I. INTRODUCTION

A. Study Objective

The purpose of NASA Contract NAS 9-12560 is to study the mechanisms of deterioration of intermediate moisture foods (IMF) and find methods to improve their stability through processing, formulation and packaging. Under Phase I and II of this contract the main problems found associated with deterioration of IMF were:

1. <u>Microbiological growth</u> - especially molds and the common pathogen <u>Staphylococcus aureus</u>.

2. <u>Non-enzymatic browning</u> - leading to off-colors and loss of protein nutritional value because of the presence of reducing sugars in most formulas.

3. <u>Rancidity</u> - leading to off-odors due to the promotion of lipid oxidation as a_w increases.

4. <u>Vitamin C deterioration</u> - due to the fact that the destruction rate increases rapidly at high a_w.

In addition to these problems, it was found that control, prediction and measurement of the product water activity (a_w) was a problem due to both lack of theoretical basis and any standards for measurement. This is increasingly important since the Food and Drug Administration has promulgated a water activity standard of 0.85 for heat processed non-acid foods and is in the process of setting a standard for cheese and cheese foods.

The major objective of this study was to develop further guidelines for formulation and processing of a shelf stable IMF that can be used as a complete meal substitute emergency food ration for the

NASA Space Shuttle program. This food would have to withstand possible high temperature, loss of package integrity without microbial safety hazard, and provide all the daily nutritional requirements.

Past work on this project has been involved in research to determine initial guidelines for the formulation and processing of a product to meet the NASA requirements. The studies in this past year of Phase III of the contract were to:

1. Determine the effectiveness of various mold inhibitors in an IM food system, especially against S. aureus at high a_w. This organisms is potentially the greatest bacterial hazard that can exist in IMF.

2. Determine the effect of a_w on the death rates of pathogenic vegetative cells in the water activity range of IMF.

3. Determine effects of food formulation on non-enzymatic browning and develop rapid methods to determine protein nutritional loss.

4. Determine effectiveness of various food grade humectants on water activity lowering and develop standard methods of a_w measurement.

5. Initiate accelerated temperature studies for evaluation of food grade antioxidants in IMF.

6. Produce an intermediate moisture cheese product within the stability guidelines suggested with respect to microbial hazards.

-11-

B. Literature Survey

  1. General Characteristics of IMF

  The introduction of intermediate moisture food (IMF)
processing in the pet foods area has caused a great surge of interest
in this type of food for human foods. In 1974 IM pet foods captured
over 40% of the multimillion dollar pet food market and the growth
potential is expanding. Because of the growth in this area much
interest has been stirred up among food companies to expand IMF
technology into the human food market.

  Several human food items made by past technology fit into
the IMF category. These include various candies, like marshmallows,
bakery goods, like fig newtons, and various sausage products. Some of
these are listed in Table 1. In most of these products salt or sugar
is added to bind the water present in the food and thus give the product
the desired stability against microorganisms without the need for
refrigeration. This stability is due to the lowering of the water
activity (a_w) of the product. In addition, since small molecules like
salt or sugar hold more water per weight than larger molecules like
starch, the product has a soft, moist texture. Using these principles,
several large food companies have developed breakfast toaster tarts of
IMF technology. These products rely on sugar and the acidity of the
fruit for shelf stability without the need for refrigeration. As with
the pet foods, these products are eaten directly. Rehydration with water
is not needed and, thus, they are very convenient to use. Table 2 lists
the general characteristics of intermediate moisture foods.

  The pet food products that have been developed are basically
a meat-sugar-cereal food which is extrusion processed at a reduced

TABLE 1

IM Foods

Food	Process
Dried fruits Soft candies Marshmallows Jams and Jellies Honey & Syrup	Sugared
Fruit cake	Baked
Pepperoni Dry salami Beef jerky	Salted & Dried
Country ham Pemmican	Dried, Salted & Sugared
Cheese	Salt, Low Water

TABLE 2

INTERMEDIATE MOISTURE FOOD CHARACTERISTICS

Moisture content -   10-40% (controllable)

a_w -   0.65 to 0.90

Directly edible -   soft moist texture
                    no rehydration
                    no refrigeration

Completely manufactured - composition can be completely controlled

Texture - hard brick to very soft pliable

Nutrient levels - can be adjusted for specific requirements

moisture content. Usually a mold inhibitor like sorbate is added since

the water activity (a_w) is not reduced to below the limits for molds.

Very little is known about the shelf stability if IM pet

foods except with reference to microorganisms. Based on the work of

Labuza (1971a) as seen in Figure 1, these foods would be expected to

deteriorate rapidly due to hydrolytic reactions, non-enzymatic browning,

and especially lipid oxidation. These reactions lead to off-odors and

off-flavors, color changes, toughening and decrease in nutritional value.

Fortunately, since the pet food products are viewed as a commodity item,

their turnover is very rapid and, thus, these deteriorative reactions

do not progress very far. In addition, with respect to rancidity,

little is known if the off-odors and off-flavors that are developed are

obejctionable to the animals. With respect to foods for humans,

however, a much longer shelf life would be required since the product

would be used most likely as a meal substitute. Several food companies

that have produced IMF type products are listed in Table 3. Some of

these products must be consumed with milk since the addition of protein

ingredients would shorten the shelf life of the products due to the

browning reaction.

Other food and drug companies are investigating intermediate

moisture foods from the standpoint of special dietary foods. In view

of the extrusion processing technology that the pet food companies use,

it is possible to completely formulate a shelf stable food for any

specific need such as for low sodium, low potassium and low protein.

The combination of these latter ingredients are very important in

chronic renal failure where, if not controlled in the diet, the patient

Figure 1. Stability Map of Foods as a Function of a_w

RELATIVE REACTION RATE
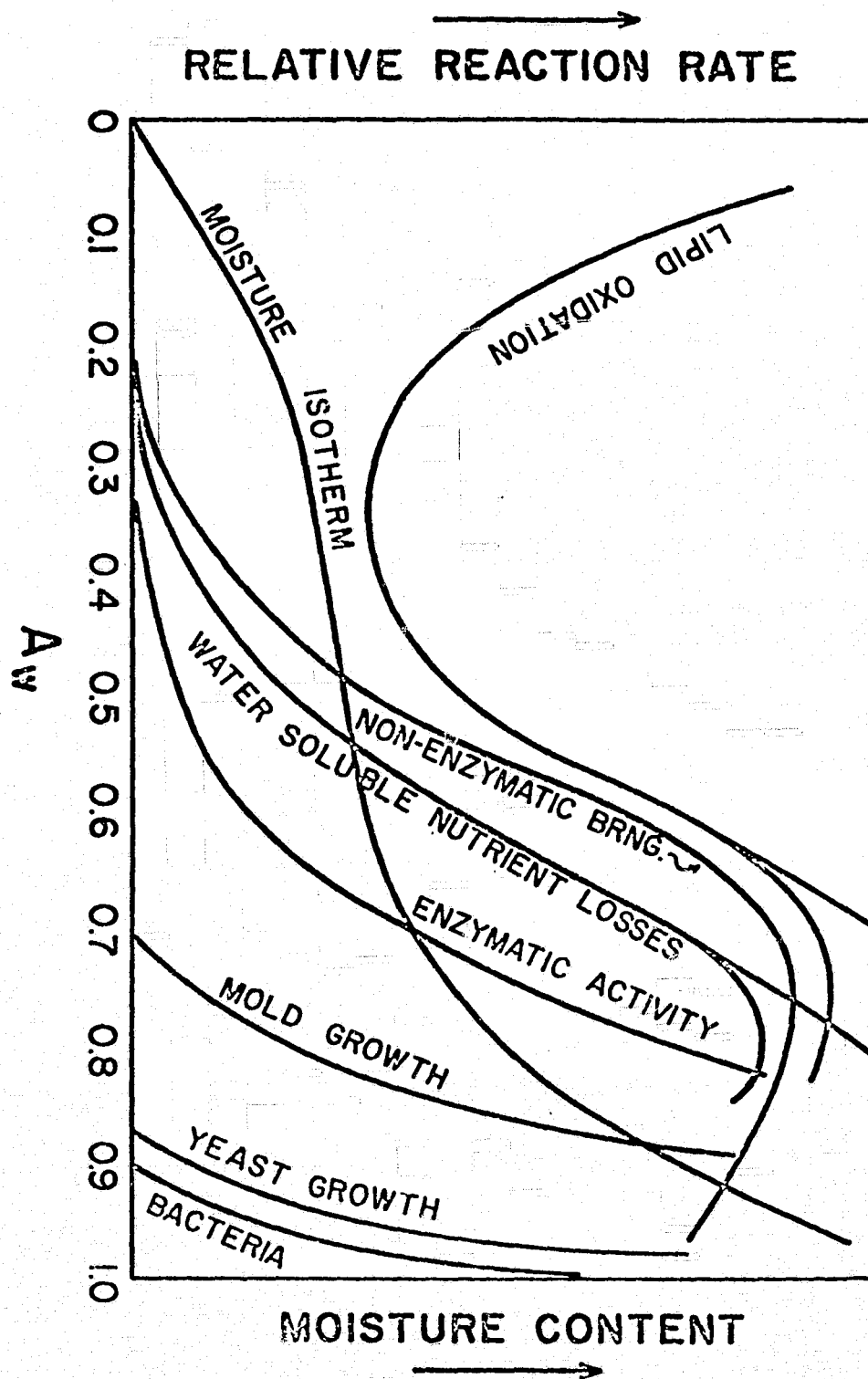
TABLE 3

HUMAN TYPE RECENT IM FOODS ON MARKET

National Biscuit Co.

Pastry Toastettes (10-12% H_2O)
Fast Break Meal Bar (consume with a
    glass of milk as meal replacer)

General Mills, Inc.

Breakfast Squares (meal replacer)

General Foods Corp.

Pastry - Toast'ems (15% H_2O)

Pillsbury Co.

Space Sticks Candy (11% H_2O)

Kelloggs

Toast'em Pop Up Pastry (13% H_2O)

Kraft

Up and At Em (test market meal replacer)

Carnation

Breakfast Bars (consume with a glass of
    milk as meal replacer)

usually dies. In addition, these people need a high caloric intake to

prevent protein catabolism which the sugar (humectant) could provide.

Even more interesting is that IM foods have a low water content.

Patients on renal dialysis usually have to be dialyzed two or three

times a week at a substantial cost which sometimes exceeds $20,000

per year. As shown by Nelson et al. (1972), if a diet is controlled

in terms of water intake, dialysis would only have to be done every

7 to 10 days at a substantial cost savings to the patient. This would

also allow dialysis units to treat more people. Thus, intermediate

moisture foods of special dietary composition should be of extreme

benefit for treatment in this disease and allow these people to have a

food that they can eat away from home.

The space program has a very big interest in an IM human

food item. NASA has supported several contracts by the present

investigator for initial development work (NAS 9-9426, NAS 9-10658 and

NAS 9-12560). An IMF item which could be highly nutritious and eaten

without hydration would be extremely desirable for the space program.

This is especially true for the space station and space shuttle where

much extra vehicular activity (EVA) could be taking place. A device

to dispense the food in pellets or cubes through the face plate or

within the helmet could be used, thus enabling the astronaut to continue

working and be supplied with the nutrients he needs.

Table 4 lists the specific benefits of using an IMF system

in the space program. These same benefits apply to military use. As

with the pet foods, these products could be eaten directly without the

need for rehydration. This is important since in the Gemini and Apollo

programs a major complaint was the problem in rehydration of the freeze-

TABLE 4

CONTRIBUTION OF IMF TO MILITARY OR SPACE PROGRAM

1. High density - low volume
2. High caloric density
3. Can be made to specific nutrient needs of each astronaut
4. Low water content - minimizes urine volume
5. No refrigeration - shelf stable
6. Completely balanced emergency ration
7. Can be adapted to any configuration
8. Can use KCl to up K load in diet without adverse effects
9. Can be used in space helmet
10. No crumbs in weightlessness
11. Can be used under stress or diversion
12. Package damage not critical

dried products and the excessive time this took for many of the foods. The ease of handling afforded by IMF products eliminates this time consumption and problems with water rehydration.

Several factors make the IMF product very suitable for military use and space flight. It is a shelf stable product, thus energy consumption for refrigeration is zero. It can be molded into any configuration thus making it suitable for carrying about easily in a uniform or for storage in minimal space. The soft, moist texture eliminates any need for rehydration thus need for a water supply is eliminated. It is a concentrated food energy source because of the lowered moisture content, and thus the weight is reduced. Lastly, it requires little preparation so can be used under duress or stress with needed diversion of attention to more important matters. With respect to nutritional benefits, because the product is completely formulated it can be matched to the needs of the military man or astronaut. Development and manufacture of an IM food should not be expensive, especially in light of the meal costs for some IM pet and human foods now on the market.

A significant problem in the production of these foods is the taste. Present procedures rely on salt, glycols and sugar to reduce the a_w of the product. The a_w lowering of solutes occurs because of Raoult's law which describes the water binding of solutes. Unfortunately, to obtain the low a_w needed (less than 0.85 to prevent food poisoning from staphylococcal organisms) high levels of salt and/or sugar are necessary. High levels are needed in spite of the fact that these compounds behave non-ideally, lowering a_w more than expected. The need

in this area is to find a humectant (a_w lowering chemical) that:

    a. is low in molecular weight

    b. if high in molecular weight then is very non-ideal,

          i.e. lowers a_w more than expected

    c. has no flavor or odor

    d. is non-toxic to humans and possibly toxic to microbes

    e. is preferably liquid and completely miscible with

          water

    f. does not react in the food to cause deterioration

    g. has a large a_w stabilizing effect

Of course, this is not the scope of the present study and certainly the

development of such a chemical would require years of animal tests.

However, it is possible that this chemical can be found and used in

the future for IMF.

The other major problem with respect to chemical stability

is the prevention of deteriorative reactions such as browning, rancidity

and nutrient losses. This current contract focuses on these as well

as the study of microbiological problems.

REFERENCES

Labuza, T.P. 1971. Properties of water as related to the keeping quality
    of foods. Proc. 3rd Int'l Congress of Food Science. Institute of
    Food Technology. pg. 618.

Nelson, R.A., C.F. Anderson, J.V. Donadia, Jr., P.P. Frohrert and W.J.
    Johnson. 1972. Water balance and frequency of hemodialysis in
    an ephric patient. Trans. Amer. Soc. Antif. Organs. 18:

2. Shelf-Life Stability Literature

In Phase I and Phase II of this contract extensive literature reviews were given which will not be reproduced in this section. Each section of this report has a literature review section pertinent to the area under consideration. In addition, a book chapter published by the principal investigator in _Theory, Determination and Control of Physical Properties of Food Materials_ (C. Rha, editor, Reidel Publishing Co., Dordrecht, Holland) is included here as a review of water sorption theory.

3. Sorption Phenomena in Foods

Reprinted on the next pages is a copy of the review article that was published in _Theory, Determination and Control of Physical Properties of Food Materials_: Proceedings of a Symposium.

SORPTION PHENOMENA IN FOODS:
THEORETICAL AND PRACTICAL ASPECTS

THEODORE P. LABUZA

Dept. of Food Science and Nutrition, University of Minnesota, St. Paul, Minn., U.S.A.

1. Definition of Water Activity – Water Content

The control of moisture content in the processing of foods by various techniques is an ancient method of preservation, but only in recent years has the physical-chemical basis of the methods been studied and understood. When food is preserved the first and foremost principle is the destruction of control of deleterious or pathogenic microorganisms. With respect to water, the methods of preservation that operate on this principle are based on the fact that the water is made unavailable for microbial growth. These methods include: salting and sugaring of foods whereby the water present is tied up in some way so as to be made unavailable; freezing by which process the liquid water is converted into a solid state and thus is removed from being available to the organisms; and drying by which process the water is removed directly by vaporization from the food material. In most of the foods after the processing, micro-biological growth is thus prevented during storage, however, chemical deterioration does occur to affect quality. As has been shown by Salwin (1959), Scott (1957) and reviewed by Labuza (1971) the relationship between the loss of quality of the food and the moisture content of the food is best represented by the term *water activity* or A_w.

The A_w defines the degree to which the water present in the food is tied up or bound and thus unavailable for certain reactions. From a physical chemistry point of view water activity is defined as: A_w is the equilibrium vapor pressure that the water in a food exerts (P_{H_2O}) divided by the vapor pressure of pure water (P_0) at the temperature of the food. Water activity also can be defined as the relative humidity of air (% RH) at which a food if held would neither gain nor lose moisture. In equation form this becomes:

$$A_w = \frac{P_{H_2O}}{P_0} = \frac{\%RH}{100} . \tag{1}$$

With respect to a food at different water activities a different amount of water is held or bound. This relationship is called the water sorption isotherm since it defines the moisture content (m) in equilibrium with the different values of A_w at constant temperature. Figure 1 shows a typical plot of these values in which moisture is cal-

culated on a grams H_2O per gram dry solids basis. Thus, at 3 g H_2O per gram solids the moisture content on a wet basis is 75%. As can be seen in Figure 1, in most foods such as fruits, vegetables, meats, fish and liquids the water activity is very high and is not too different from that of pure water. It is not until the moisture content is reduced to less than 50% on a wet basis does the A_w get reduced. This lowering is due to many factors which will be discussed subsequently. Firstly, the methods of determination of the water sorption isotherm should be examined.
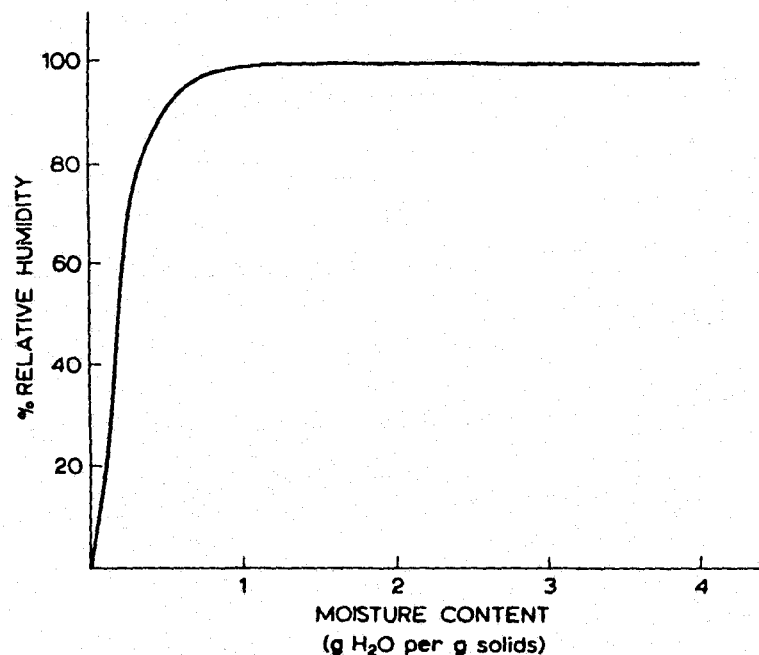
Fig. 1.   Water content – water activity of foods.

2. Measurement

Figure 2 shows the general shape of the water sorption isotherm for foods. It is just the expansion of the region in which A_w is lowered from Figure 1. As seen it represents the moisture content in equilibrium at each A_w. The curve also shows hysteresis, i.e., the equilibrium moisture at a given A_w depends on the direction the isotherm was made from. A higher m is usually observed as one does the experiments from the desorption direction by drying out the food as opposed to humidification.

In order to establish the isotherm both A_w and moisture content must be determined. Many methods are available; the important ones are discussed below. It should be noted, as the term isotherm implies, it is assumed that the values are established for the product at some constant temperature.

2.1. Moisture

Moisture content means the determination of the water held in the food. Since the water is bound by some energy either one must apply this energy to remove the water from the food and measure the weight change or measure the energy level by some electronic technique. Each method used gives a different basis making comparisons between various researchers sometimes difficult.
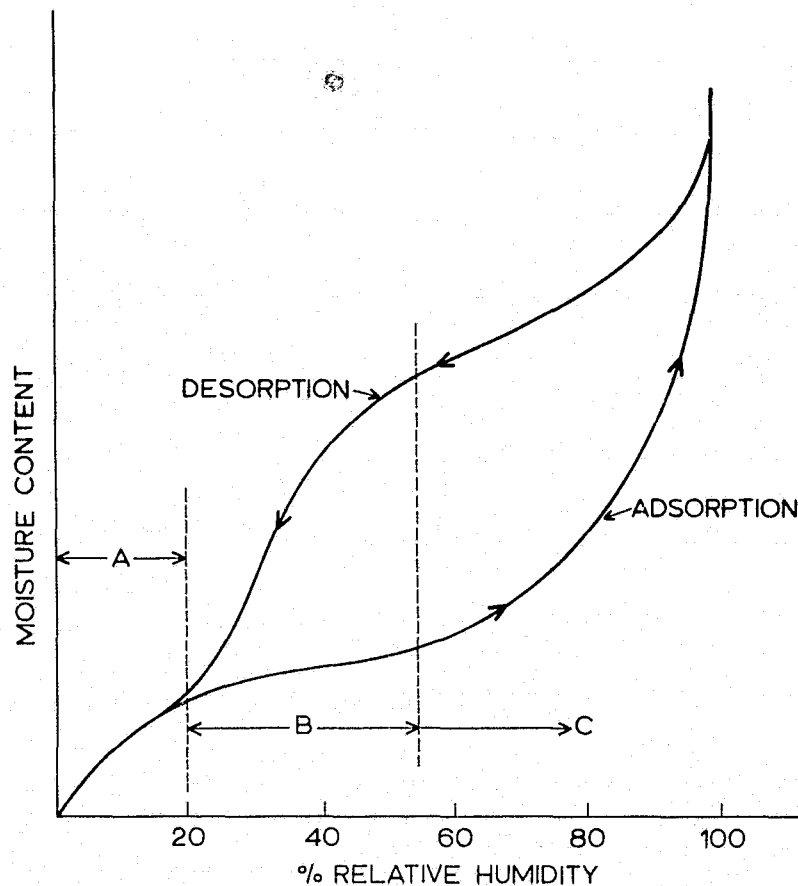
Fig. 2. Typical sorption isotherm of a food showing hysteresis.

In terms of weight change techniques, most of the common methods involve drying the food at some temperature for a specified time period. The air oven technique is probably the most crude of these methods and consists of placing the product in a circulating (or noncirculating) air chamber at a relatively high temperature (about 100–200°C) for 18 to 48 h. The A.O.A.C. has set specifications for various foods by this technique. The basic problem is that the high temperature can cause reactions which could change the weight of the food, volatiles may be lost, and the method is

destructive to the sample. The method is also relatively slow: even after 24 h the weight may still be changing. The precision of the method is based on the precision of the weighing device used and one must weight both before and after. The accuracy will vary due to heterogeneity within the sample, the sample size, and the time used.

The vacuum oven technique overcomes some of the problems by using a lower temperature (60–70 °C) and drying the sample in a vacuum so that the rate of diffusion of water is faster. However, volatile loss would still occur reducing the accuracy of the technique. In industry infrared oven devices are used quite extensively for moisture determination. Many companies manufacture these devices which are basically enclosed balances on which the sample is tared and then dried in 1 to 20 min by a heat lamp. Usually the temperature is over 400 °F. This gives a tremendous error in accuracy, but as a daily quality control device for the same type of food such as meat emulsions it is useful. It should not be used for isotherm determinations.

A very accurate technique is to use freeze-drying at room temperature. It has the advantage of the lower vacuum to speed the rate of drying, but since a temperature of 20–25 °C is used to prevent volatile loss it takes at least 24–48 h. Again, the sample must be weighed before and after. The use of P_2O_5 desiccant is one of the most accurate techniques but takes a long time (5–7 days). It is based on the fact that P_2O_5 has a high absorptive capacity for water with a very low vapor pressure. The technique uses the low vapor pressure of P_2O_5 instead of heating the air to a low vapor pressure or using a condensor as in freeze-drying. Since there is no hot air circulating over the sample, or a vacuum continuously being pulled, little or no volatiles are lost making it the most accurate technique.

Other methods are based on extracting or volatilizing the water in the food by means of an organic solvent. One technique, toluene distillation, is based on mixing the food with toluene and then boiling the mixture. The water is removed at a lowered temperature by steam distillation with the toluene. The method is rapid but is not very accurate. It is used basically for quality control techniques. Much more accurate is the Karl Fisher Technique in which the food is extracted with methanol. The extraction is done in an oxygen-free atmosphere and the mixture is titrated with a reagent (sulfite) to measure the water. Many rapid technique devices have been built for this test which require about 20 min per sample. The basic theory is that the methanol replaces the water bound to the food, however, not all of it is replaced so the method is not as accurate as the P_2O_5 technique. However, it is fast even when done at room temperature. The other problems that occur however are interfering reactions of the reagents with food components and the extremely obnoxious odor of the sulfite reagent.

To eliminate these problems the methanol extract (after $\frac{1}{2}$ to 1 h) can be injected directly into a gas chromatograph using Pora-Pak Q as a column packing. The water is separated and detected by a thermal conductivity detector. This method is accurate and rapid and is being used by many researchers and companies.

Other methods are based on the physical-chemical properties of bound and free water. Sudhakar et al. (1970) has documented the use of NMR techniques for measuring water. Many other instruments devised for moisture content are measurement of

dielectric strength, absorption spectroscopy, and infrared spectroscopy. These techniques besides giving some information as to the structure of water in foods can also be adapted to on-line process control of moisture content without destruction of the product. It should be remembered, however, that all these methods require that the instrument be calibrated by some other moisture measurement technique. Therefore any inherent error in the moisture determination is carried over into the instrument reading. A review of the various moisture determination techniques has been made also by Heiss (1968), Hofer and Mohler (1962), Gal (1967) and Stitt (1958).

2.2. WATER ACTIVITY

As with moisture content, many techniques both direct and indirect are available for the measurement of water activity. The oldest technique, that of the hair hygrometer was in fact reported by Leonardo da Vinci (*Hygrodynamics Technical Bulletin* #4). A hair being basically a protein will absorb water and change in both weight and length as the humidity increases. The hair can be tied to a pointer to indicate the degree of saturation of the vapor space in terms of % relative humidity. The device is very inaccuarte below 30% and above 80% RH but is used (usually with synthetic fibers) in home air conditioning units for humidity control. It is not accurate for research purposes.

A very accurate technique which cannot be applied to A_w measurement of small samples is wet bulb psychrometry. The method is described in detail by Van Arsdel (1963) and involves spinning two thermometers, one of which is immersed in a wet wick, in the vapor space. Based on the properties of the air the relative humidity can be determined. This technique was used by the U.S. National Weather Service. It is still used in the measurement of the % RH in large warehouses such as in potato sheds where there is a large air volume. The method is fast but cannot be applied to small samples. However, a dew point device employing the same principle can be used. In this case a surface is cooled and the temperature at which water condenses is measured optically. This then can be related to the A_w. Instruments available have an accuracy of $\pm 3\%$ RH.

One of the best direct measurements of A_w is the direct measurement of the vapor pressure of water in the vapor space surrounding the food by manometric techniques. Devices based on this have been described by Taylor (1961), Karel and Nickerson (1964) and Labuza *et al.* (1972). The device as shown in Figure 3 consists of a sample flask connected to a manometer. The system is thoroughly evacuated (to less than 200 μ Hg) with the vacuum unconnected to the sample. The vapor space around the food is then evacuated for less than two minutes to remove the gases present and then the stopcock across the manometer is closed. The whole system is kept at constant temperature and the food sample will lose water to reequilibrate with the vapor space. This will be indicated by the difference in height on the manometer. A low density, low vapor pressure oil should be used for maximum precision. The sample size to vapor space should be large so that a loss in water from the food will not change its A_w or moisture centent significantly. After equilibration, usually 40–60 minutes for a

-27-

5-10 g sample, the stopcock to the sample is closed, the one to the desiccant flask is opened and after 30 min the difference in oil manometer height indicates any leaks into the system as well as volatiles or gases lost from the sample. This system is extremely accurate ($\pm 0.002\ A_w$ units) as long as temperature is accurately controlled. Liquid samples usually should be frozen first and, therefore, require more equilibration time as they warm up.

Another technique which directly measures water in the vapor space is the use of gas-liquid chromatography (GLC). A sample is equilibrated in a jar, and an air sample
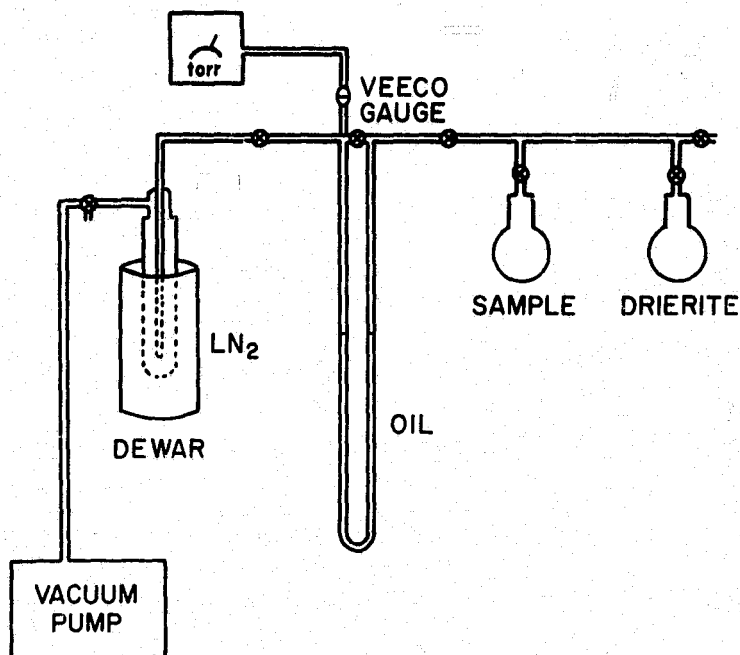
Fig. 3. Manometric device for measuring A_w.

is treated similarly to the liquid methanol-water determination method. Unfortunately, at high A_w the suction and compression of the gas tight syringe can cause condensation of water vapor leading to inaccuracies.

Electrical devices have been designed to do the same thing indirectly. Schmidhofer and Egli (1972) have described the use of the Sina-Scope for A_w measurement of foods. This measures the absorption of water vapor on a filament and can be directly related to water activity in the vapor space. This device is expensive and is not used by many researchers. The most used devices are the relative humidity sensors based on electrical resistance. A probe which contains a filament coated with a salt is placed in a chamber containing the food. After equilibration, usually 1 h to 24 h, a current is passed through the filament and a readout is made on a dial. Depending on the probe

-28-

used, the filament salts absorb different amounts of water and thus the resistance to current flow changes. A chart then is used to convert the readout to % RH or A_w. Although good to within ±0.5% RH when new, the probes age and become less accurate so they must be recalibrated. They also are subject to errors due to absorption of volatiles from the food such as glycerol. These types of instruments should not be used for accurate research work, but since they are relatively inexpensive, they are useful for quality control and on-line measurements.

The last direct method of A_w measurement is by measurement of freezing point depression as described by Strong et al. (1970). This can only be used for high A_w systems (low solute concentration) and not for solid foods. It is based on the Clasius Clayperon Equation for dilute systems and assumes the activity coefficient to be equal to one (Equation (2)). This method is used basically by microbiologists for studies of microbial growth as a function of A_w when mixed solute systems are used as media.

$$n_2 = \frac{\text{grams solvent}}{1000} \cdot \frac{\Delta T_f}{1.86} \tag{2}$$

$$A_w = \gamma \frac{n_1}{n_1 + n_2}, \tag{3}$$

where

$n_1 = $ moles of water in system,
$n_2 = $ moles of theoretical solute in system
$\Delta T_f = $ measured freezing point depression °C
$\gamma = $ activity coefficient $= 1$.

Because of either the expense of equipment or difficulty in operation most researchers use the indirect method of controlling water activity. This is done by using a chamber system in which the A_w can be directly controlled by some means. The moisture content after equilibrium is then measured rather than the water activity as it is a much simpler technique. The basic technique is the use of a saturated salt solution slurry. In a desiccator various salts at saturation give a definite A_w which varies little with temperature. Tables of salt solutions vs. A_w have been compiled by Wink and Sears (1950), Richardson et al. (1955), Rockland (1960) and in the *Hygrodynamics Technical Bulletin* #5. Table I lists some of the more common salt systems used. The method has some problems however:

(a) The salt must be saturated at the temperature of the experiment.

(b) There must be a large surface area and small air space volume available to the adsorbing or desorbing sample.

(c) The vapor space should be the same temperature as the liquid (a 2°F difference can, for example, lead to a 5% RH error).

(d) The salts and water used must be pure.

(e) The solution should be a slurry since if the sample loses water it will dissolve fresh salt. If the sample gains water the surface should not dry out.

(f) Salts are not available to give a point at every A_w desired.

To get around the latter problem both glycerol-H_2O and sulfuric acid-H_2O mixtures have been used. These allow for a continuous number of water activities but since at low A_w if the samples lose or gain water the constant humidity solution changes composition and therefore the A_w changes. This can be compensated for by using a large solution volume to sample volume and also measuring the specific gravity of the solution afterwards to determine the equilibrium A_w. Lastly, a mechanical chamber, of which there are many, can be used to provide a constant humidity. The better the

TABLE 1

Typical salts used for
constant humidities 20°C

Salt used	A_w
Desiccant	< 0.001
$LiCl_2$	0.12
$MgCl_2$	0.34
K_2CO_3	0.49
$Mg(NO_3)_2$	0.55
$NaNO_2$	0.65
NaCl	0.76
$CdCl_2$	0.82
K_2CrO_4	0.88
KNO_3	0.94
Na_2HPO_4	0.99

accuracy the more expensive the device with prices going over $ 5000. The latter devices are rarely used because a chamber would be needed for each A_w. With the salt solutions inexpensive desiccators or even jars can be used. Most researchers use vacuum desiccators which allow equilibrium in under 24 h. Thus, with a number of desiccators a complete isotherm can be made in one day. Some anomalous results can occur, however, if nitrite or halogen salts are used (Chou and Labuza, 1972).

3. Factors Responsible for Lowering of Water Activity

The physical-chemical factors responsible for the lowering of A_w have been reviewed extensively by Heiss (1967), Van Arsdel (1963), Labuza (1968) and Labuza (1971). The important principles should be reemphasized as it explains the fundamental interactions of water within a food in terms of storage stability.

3.1. SOLUTE-WATER INTERACTIONS

The basic factor lowering A_w when a solute such as sodium chloride is dissolved in water is the fact that these solutes associate with the water to form a hydration shell. Depending on the amount present, the availability or vapor pressure of water is decreased according to Equation (3). Most solutes, especially as concentration is increased, behave non-ideally and bind or structure water more than predicted. This is

true for solutes of importance to the food industry such as salt and sugar. Home (1969) has listed the actual A_w for various solute solutions and has pointed out the extreme non-ideal behavior of some large molecular weight molecules. Table II summarizes some of the typical A_w values for salt solutions. Sucrose and sodium chloride are ideal substances for use in making intermediate moisture foods (those with an A_w of 0.80 to 0.88), however, the amount that can be used is limited by their taste in the product. A mixture is usually used therefore.

TABLE II

Water activities of solutions of common
salt and sugar for use in
intermediate moisture foods

Salt	Solution concentration by weight	A_w
Sodium chloride	3%	0.98
	5%	0.97
	10%	0.93
Sucrose	5%	0.999
	10%	0.994
	20%	0.993
	40%	0.96
	60%	0.89

With any solid system a second important factor in lowering A_w is the capillary effect in a food. According to the Kelvin Equation (Equation (4)), the vapor pressure or

$$A_w = \exp\left[-\frac{2\gamma \cos\theta\, v}{rRT} \right], \tag{4}$$

where

γ = surface tension of liquid in capillary,

θ = contant angle,

v = molar volume of liquid,

r = capillary radius,

R = gas constant,

T = absolute temperature,

activity of a liquid present in a capillary is reduced as the radius of the capillary decreases. For pure water the values in Table III have been calculated. As seen, the effect becomes important only in capillaries of a radius less than 1000 Å. Bluestein and Labuza (1972) have shown that most of the capillaries in a food are of greater than 10 μ size but as water is removed the water present in small capillaries (<100 Å) comprises a significant amount of the total water. Thus, these capillaries do control partially the lowering of A_w. The actual contribution is difficult to assess, however,

since the Kelvin Equation is inconsistent at low capillary size (Shereshefsky *et al.*, 1950) and the values of γ and θ for pure water cannot be used in foods (Salas and Labuza, 1968; Labuza and Rutman, 1968; Labuza and Simon, 1969).

A third factor responsible for lowering of water activity can account partially for the fact that hysteresis occurs. A different path is followed depending on whether the isotherm is approached by adsorption or desorption. Labuza (1968) and Gregg and Sing (1967) have reviewed some of the basic reasons for hysteresis, however, another possibility not presented previously is the supersaturation of solutes as moisture is

TABLE III

Effect of capillary size
on lowering A_w

Radius		A_w
μ	Å	
0.1	1000	0.99
0.01	100	0.91
0.001	10	0.89

decreased, i.e., the solutes, as they lose water, form a glass that holds more water than expected and so not crystallize out at the true A_w of crystallization but must be dried to a lower A_w. This also suggests that the desorption branch is not the true equilibrium. This principle is illustrated in Figure 4.

The last and most important factor in controlling the vapor pressure of water is the interaction of water with solid surfaces as well as with high molecular weight colloidal systems. Water molecules usually interact with the polar groups on surfaces and are held very tightly. The energy to remove these water molecules is greater than the energy to vaporize a water molecule from the surface of pure water. If one starts from a dry state, a moisture content-A_w is approached where there is one water bound per polar group to form a monolayer (McLaren and Rowen, 1952). This occurs close to the inflection on the isotherm which gives a monolayer value of about 0.2 A_w to 0.3 A_w for most foods. Water above this monolayer is usually thought of as being the same as pure water, but long-range effects do occur which structure the water in such a fashion that it is also 'bound' to a certain degree to have a lower A_w. Ling (1965) has studied this effect in living tissues and Nemethy (1968) calculated the effect of large molecules such as proteins on structuring water in solutions. Duckworth (1972) recently demonstrated that large molecules by themselves can prevent water from freezing at temperatures down to $-20\,°C$. This was shown to be directly related to the A_w lowering effect on the water.

In summary, there are many aspects of a food system which causes the lowering of the availability or activity of water. At normal tissue moisture these effects are very small, but as moisture is removed the ratio of solid to liquid increases and the

water activity is greatly reduced. Many people have tried to quantitate the degree to which water is bound or free in a food, however, as shown by Labuza et al. (1970) and Labuza (1971) water even below an $A_w = 1$ still has the properties of that of bulk water. This water can still dissolve solutes, act as a medium for their mobilization, allow reactions to occur within the structure itself and be available as a reactant for reactions such as hydrolysis.
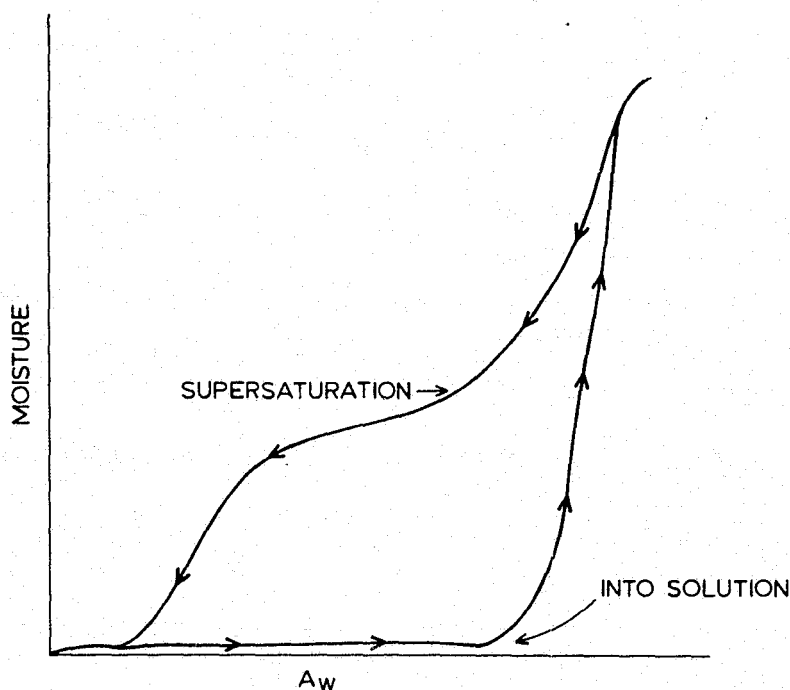
Fig. 4. Water binding effect due to supersaturation of solutes and hydrate formation.

4. Isotherm Equations

Many theories have been developed to describe the shape of the sorption isotherm both from a theoretical and a mathematical standpoint. The need for an equation is obvious within food processing operations such as in drying, shelf life predictions, storage moisture content for maximum stability, etc. Adamson (1960) and Gregg and Sing (1967) have reviewed the theoretical basis of the major isotherm equations. Labuza (1968) has discussed the use of these equations within the food field. The basis of these equations will not be discussed here; the reader is referred to the above references. It should be noted that most equations are usually based on fitting a theory to a sigmoid curve and as Gregg and Sing (1967) have pointed out, most of the thories fall apart if one tries to apply them to the sorption of water. The major isotherm equations will be presented below.

4.1. BET ISOTHERM (Brunauer et al., 1938)

The BET isotherm is the most popular isotherm of use in the food field as it gives very simply the value of the monolayer of water sorption. This is important since as shown by Salwin (1959), Rockland (1957) and Labuza (1971) the monolayer value of moisture is the moisture content at which the food is most stable. The basic equation was derived from the kinetic gas theory and has the form of:

$$\frac{A_w}{(1 - A_w)\, m} = \frac{1}{m_0 c} + \frac{(c - 1)\, A_w}{m_0 c},$$

(5)

where

$m =$ moisture content at given A_w,

$m_0 =$ monolayer value,

$c =$ constant.

Plotting the left-hand side of Equation (5) vs A_w as in Figure 5 gives a straight line for values up to A_w 0.3–0.5. Above that the theory no longer holds, but since three or four isotherm points can be determined below the maximum using the saturated salt solution method this is quite useful for foods.
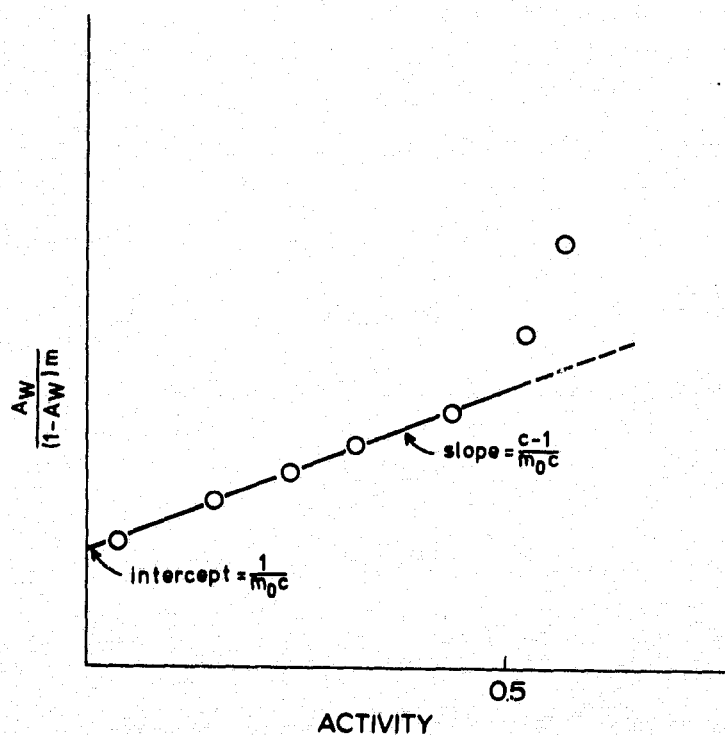
Fig. 5. BET isotherm plot.

4.2. HARKINS JURA ISOTHERM (Harkins and Jura, 1944)

Many people have advocated the use of this isotherm since it is simpler than the BET equation. The basic form of the equation is as in Equation (6) which is based on the two dimensional gas theory.

$$\ln A_w = B - Am^{-2}, \tag{6}$$

where A and B are constants.
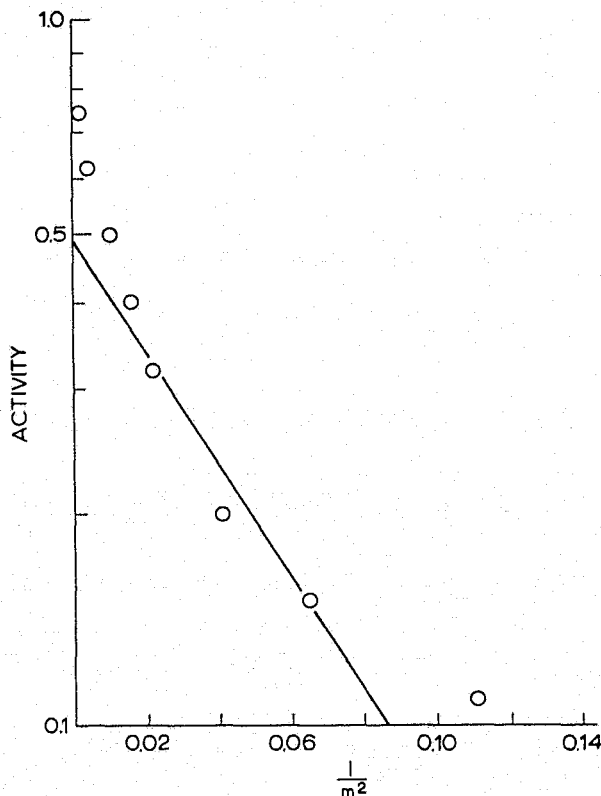
Fig. 6. Harkins-Jura isotherm plot.

Plotting $\ln a$ vs m^{-2} should give a straight line (Figure 6) but in fact since the theory only applies up to a monolayer a curved line results. From the value of A (the slope of the line) the monolayer moisture can be determined by Equation (7). The basic problem is that most of those who use the

$$m_0 = 10^{-2} A^{1/2} \tag{7}$$

isotherm apply it over the whole range of the isotherm as in Figure 6 which is theoretically impossible (Kapsalis *et al.*, 1968).

4.3. CAPILLARY CONDENSATION ISOTHERM (Zsigmondy, 1911)

Use of the Kelvin Equation (Equation (4)) has been advocated for the isotherm equation, however, all the problems discussed previously with respect to the properties of water and capillary size tend to make it unacceptable. Even of more difficulty is the fact that there has been no food for which a true pore size distribution has been determined.

4.4. OTHER EQUATIONS

Several other equations have been used for isotherm description both based on theoretical as well as empirical derivations. Rockland (1957) has favored the Henderson Isotherm (Equation (8)) based on the kinetic gas law and Kuhn (1964) has derived a general isotherm (Equation (9)). Labuza *et al.* (1972b) have used a general linear equation (Equation (10)), a Pearson's Series Expansion as derived by Oswin (1946) (Equation (11)) and a purely statistical equation (Equation (12)).

$$\ln(1 - A_w) = Am^B \tag{8}$$

$$m = \frac{A}{\ln(1/A_w)} + B, \tag{9}$$

$$m = BA_w, \tag{10}$$

$$m = A\left[\frac{A_w}{1 - A_w}\right]^B, \tag{11}$$

$$A_w = \frac{A + m}{B + m}, \tag{12}$$

where A and B are constants derived by curve fitting.

The latter three equations are most amenable to use in mathematical solutions for drying and packaging because they can be easily integrated in unsteady state transport equations.

5. Practical Applications of Food Isotherms

5.1. STORAGE STABILITY

As stated previously, the moisture content-water activity of a food can be used to predict the storage stability of a food. The basis for this has been reviewed by Labuza *et al.* (1970) and Labuza (1971) from the standpoint of the solvent properties of water and the degree to which it is bound in food.

The control of water content of a food is a basic food processing technique. It is based on the fact that the water content is decreased to a level to which microbial growth is prevented. This can be done by drying which completely removes the water or freezing which converts the liquid water into a solid state both of which make the liquid water unavailable for microbial growth. Water does not have to be completely removed, however, and as in the process of salting or sugaring which are age old

processes, and in the new intermediate moisture food technology, chemical agents are added which bind the water to make it unavailable for microbial growth. This binding in fact is measured by the degree to which the A_w is lowered. Table IV lists the water activities below which common micro-organisms are inhibited in growth. Much work is needed in the area of the interaction of, for example, heat processing and A_w (Murrel and Scott, 1966), the interaction with pH, and the interaction with antimetabolites.

TABLE IV[a]

A_w growth minima for micro-organisms

A_w	Bacteria	Yeast	Molds
0.96	Pseudomonas		
0.95	Salmonella		
	Eschericia		
	Bacillus		
	Clostridium		
0.94	Lactobacillus		
	Pedioccus		
	Microbacterium		
0.93			Rhizopus, Mucor
0.92		Rhodotorula	
		Pichia	
0.90	Micrococcus	Saccharomyces	
		Hansenula	
0.88		Candida, Torulopsis	Cladosporium
0.87		Debaryomyces	
0.86	Staphylococcus		
0.85			Penicillium
0.75	Halophilic bacteria		
0.65			Aspergillus
0.62		Zygosaccharomyces	
0.60			Xeromyces

[a] adapted from Leistner (1970).

If microbiological problems are eliminated by control of A_w the storage life of a food becomes limited due to chemical reactions. The rates of these reactions can be predicted as a function of water acitivity of the food. For example, below the mono-layer moisture content value, very few reactions can proceed which require the solu-bilization of reactants and an aqueous phase for reaction. This is because water is tightly bound at the monolayer and does not behave as bulk water (Duckworth and Smith, 1961).

Above the monolayer hydrolytic reactions increase with increasing A_w. Reactions of importance that have been studied are enzyme hydrolysis (Acker, 1969) chlorophyll degredation (LaJolla et al., 1972), sucrose hydrolysis (Schobell, 1969), anthocyanin destruction (Erlandson and Wrolstad, 1972) and ascorbic acid destruction (Karel and Nickerson, 1964). In all cases it can be shown that above the monolayer, the water, even though still having a low A_w, is available as a solvent, solubilizer and reactant.

Thus, for prevention of these reactions it is best to keep dehydrated foods as close to the monolayer A_w as possible.

A more complicated reaction that occurs in storage of dehydrated foods is that of non-enzymatic browning through the Maillard Reaction. This is a reaction of reducing sugars under the influence of either free amino acids or protein side chains leading to darkening, off flavor and loss of solubility of proteins. For long-term storage this also
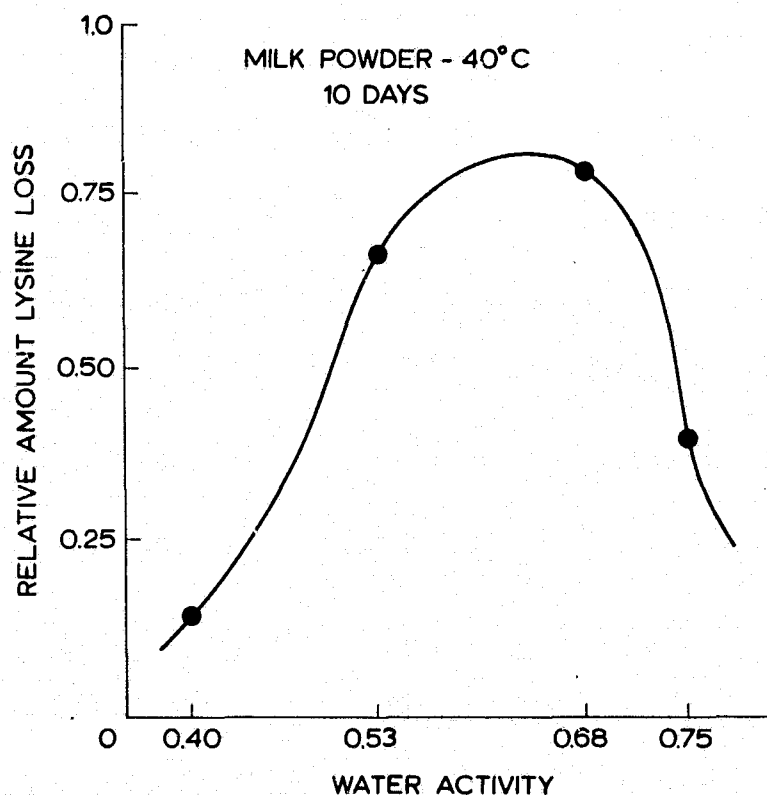
Fig. 7. Lysine loss due to non-enzymatic browning of milk powder.

means a reduction in the biological value of the food since lysine, an essential amino acid, becomes tied up to the pigment produced in the reaction. Water plays a very important role in the non-enzymatic browning reaction in that it dissolves the substrates and mobilizes them for reaction. The reaction usually does not occur below the monolayer and proceeds at a linear rate at constant A_w. A maximum in rate occurs, however, at an intermediate moisture as is illustrated in Figure 7 from the data of Loncin et al. (1968) for lysine loss. This maximum is attributed to the fact that at some A_w a greater increase in moisture content causes a decrease in the dissolved solute concentration leading to a lowering of the browning rate. Eichner and Karel (1972)

-38-

Lipid Oxidation
Unsaturated fatty acid or triglyceride

Free Radicals → Oxidation of Pigments, Flavors, and Vitamins

+ Oxygen

Hydroperoxides

Breakdown Products | Polymerization | Insolubilization
(including rancid) | (Dark Color) | of proteins
off-flavor compounds) | (possibly toxic)

such as, ketones
aldehydes
alcohols
hydrocarbons
acids
epoxides
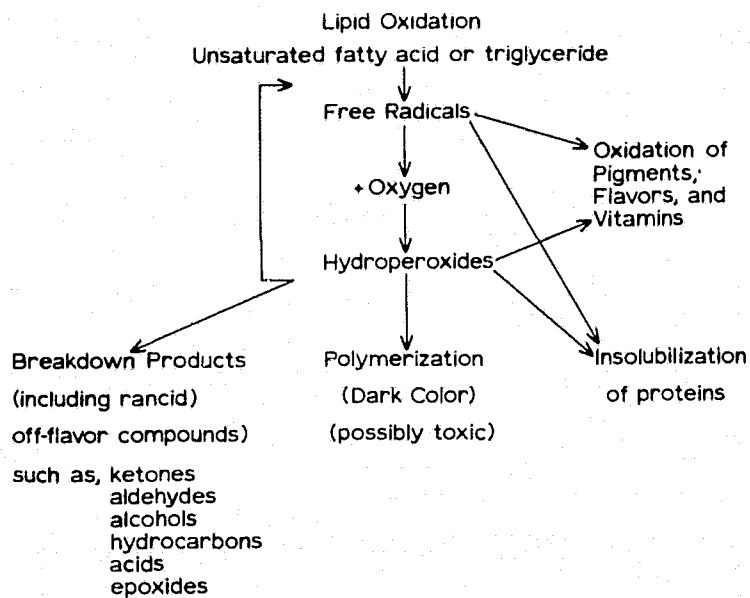
Fig. 8. Lipid oxidation pathway.
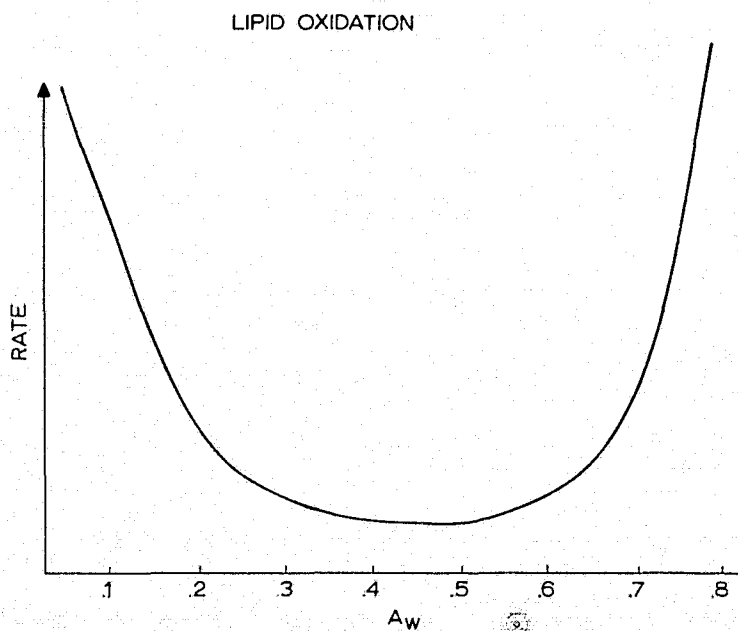
LIPID OXIDATION

Fig. 9. Effect of A_w on lipid oxidation rate.

has also shown this to be due to product inhibition by water since water is a product of the browning reaction.

Another major reaction limiting storage stability of dehydrated foods is lipid oxidation. The major pathways of lipid oxidation are shown in Figure 8 and the general influence of water activity is illustrated in Figure 9. As seen, moisture content once again plays a major role in controlling the oxidation of lipids with a minimum in rate occurring somewhat above the monolayer. Below the monolayer A_w, the rate increases rapidly as it also does in the intermediate moisture range. Since other reactions, as discussed above, also increase in rate above the monolayer, this A_w is indicated at the best condition for maximum storage stability of dehydrated foods.

With respect to lipid oxidation, the reason for the minimum curve (Figure 9) can be explained on the basis of the interaction of water in the system. Water has both antioxidant and prooxidant properties. At low A_w as moisture increases it hydrates metal catalysts making them less active and hydrogen bonds with the peroxides produced taking them out of the reaction. Thus, the rate decreases as A_w increases (Labuza et al., 1969). At a certain A_w above the monolayer, however, these catalysts become more mobile and even though they are less active their effective concentration is greater and thus the rate increases (Heidelbaugh and Karel, 1970). New catalysts may also be dissolved to enhance the reaction. In addition, swelling of the polymeric matrix of the food should open up new capillaries making more catalyst sites available for reaction (Chou et al., 1972). In some cases a maximum should occur in the intermediate moisture range if swelling is not important since increased moisture would dilute the metal catalyst concentration (NASA Contract Report 9–12560, November 1972).

Overall, as illustrated in Figure 10, a stability map can be drawn which directly relates the stability of a food to its A_w. Once this is known for a particular food and the isotherm is determined then as shown by Salwin and Slawson (1959) one can predict 'a priori' what would be the ideal combination of ingredients in making a dried food mixture such as for soup.

5.2. PACKAGING PREDICTIONS

A unique use of the isotherm equations and the storage stability map has been made by the research group from MIT in a series of publications (Mizrahi et al., 1970a, 1970b; Simon et al., 1970; Labuza et al., 1972; Quast and Karel, 1972; Quast et al., 1972) on the prediction of the shelf life of foods stored in semipermeable films. In these studies it was shown how to use reaction kinetics of deterioration as determined under steady state conditions and apply them to the condition where moisture is being slowly transported into a package. The food is thus continually increasing in moisture or A_w, if the instantaneous reaction rate for deterioration is known then the amount of deterioration can be summed up. The only other factor that has to be determined is the cutoff value. This is the amount of reaction that is allowed up to the point of unacceptability. Thus, the total time to reach that value can be predicted.
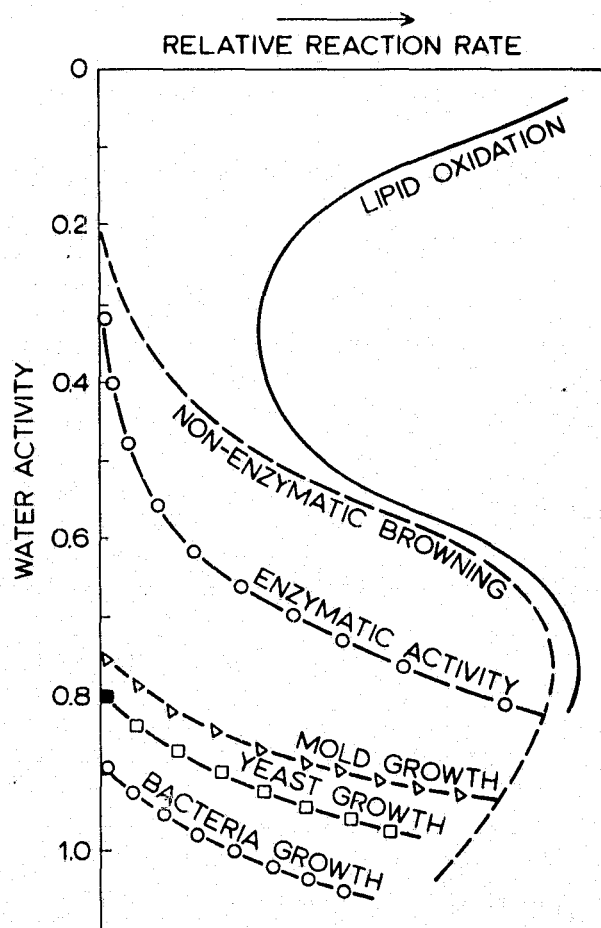
Fig. 10. Stability map for dehydrated and intermediate moisture foods.

As a simple example one can take the case of moisture adsorption into a package of grain. One limiting acceptability factor would be the critical water activity at which mold would grow on the grain. This A_w could be related to the food through the isotherm to give a critical moisture content. The rate at which the package of food picks up water is:

$$\frac{dw}{dt} = \frac{k}{x} A (P_{out} - P_{in}),$$

(13)

where

w = weight of water gained by package,
k = package permeability to water,
x = film thickness,
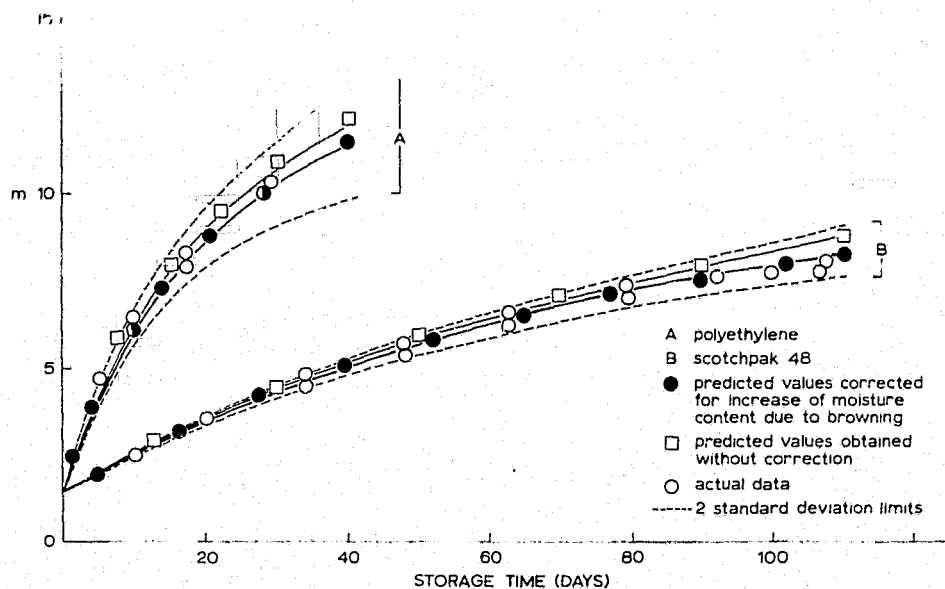
-Comparison of predicted increases in moisture content of samples stored in 2 types of packages, and experimental results
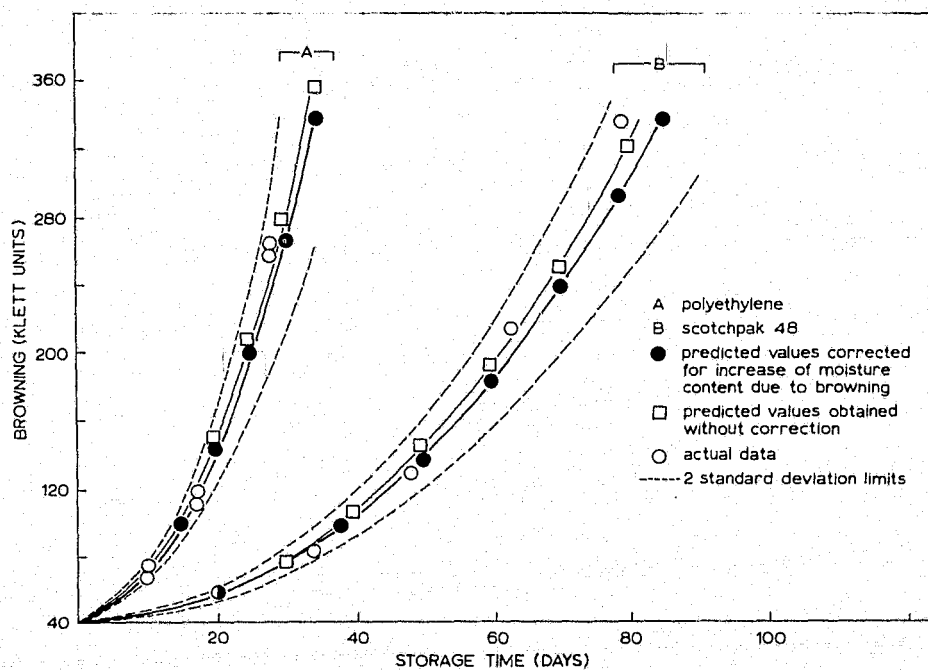
-Comparison of predicted increases in browning of samples stored in 2 types of packages, and experimental results.

Fig. 11.   Moisture and browning predictions for dehydrated cabbage.

A = film area,

P_{out} = outside water vapor pressure,

P_{in} = vapor pressure of water in equilibrium with the food.

If the assumption is made that the major resistance to water vapor flow is the film, then the water in the package should equilibrate rapidly. Thus, P_{in} can be determined from the isotherm. Simply, if one assumes a linear isotherm them:

$$m_{in} = BP_{in}/P_0, \tag{14}$$

where m_{in} is the instantaneous moisture content of the food. Also, the value of P_{out} is related to the equilibrium moisture content of the food if left in the outside atmosphere with no package. Dividing both sides of Equation (13) by w_s, the weight of dry solids, and substituting for the linear isotherm we get:

$$\frac{dm}{dt} = \frac{k\ A\ P_0}{x\ w_s\ B}(m_e - m) \tag{15}$$

which when integrated gives:

$$\phi = \frac{k\ A}{x\ w_s} = \frac{B}{P_0 \theta_c}\ln\frac{m_e - m_i}{m_e - m_c},$$

where

m_c = outside equilibrium moisture content,

m_i = initial moisture content,

θ_c = critical shelf life desired.

In this form one can, by substituting in the moisture values, the isotherm slope (B) and the shelf life desired, get the value of ϕ necessary to reach those conditions. For a given weight of food (w_s) and package size (A) thus one could get the film permeability and thickness necessary for this. Then one could choose a film that does this at the least expensive cost. This methodology works and has been verified by a number of food companies.

For more complicated reactions such as browning the methodology referenced above should be studied. Figure 11 shows a study made by Mizrahi et al. (1970a), for example, in which both the predicted moisture increase and predicted browning come very close to the actual values.

6. Summary

The importance of the water activity-moisture content concept of foods cannot be over emphasized. This paper has tried to summarize some of the approaches to understand and measure these values. More importantly, the value of water activity has been shown to control the stability of dehydrated and semi-moist foods.

References

Acker, L. W.: 1969, 'Water Activity and Enzyme Activity', *Food Tech.* 23, 1241.

Adamson, A.: 1960, *Physical Chemistry of Surfaces*, J. Wiley & Sons, New York.

Bone, D.: 1969, 'Water Activity: Its Chemistry and Applications', *Food Prod. Devel.* (August) 81.

Brunauer, S., Emmett, P. H., and Teller, E.: 1938, 'Adsorption of Gases in Multimolecular Layers', *J. Am. Chem. Soc.* 60, 309.

Chou, H. E. and Labuza, T. P.: 1972, 'Anamolous Effects of Humidity Control', *J. Ag. Food Chem.* 20, 1288.

Chou, H. E., Acott, K., and Labuza, T. P.: 1972, 'Sorption Hysteresis and Chemical Reactivity: Lipid Oxidation', *J. Food Sci.* 38, 316.

Duckworth, R. B.: 1972, 'Properties of Water around Surfaces of Food Colloids', Dept. of Food Science. University of Strathclyde. Scotland.

Duckworth, R. B. and Smith, G.: 1963, 'Environment for Chemical Change in Dried and Frozen Foods', *Proc. Nutr. Soc.* 22, 182.

Eichner, K. and Karel, M.: 1972, 'Influence of Water Content and Water Activity on the Sugar-Amino Browning Reaction in Model Systems', *J. Ag. Food Chem.* 20, 218.

Erlandson, J. A. and Wrolstad, R. E.: 1972, 'Degradation of Anthocyanins at Limited Water Concentration', *J. Food Sci.* 37, 592.

Gal, S.: 1967, *Die Methodik der Wasserdampf-Sorptions-Messungen*, Springer-Verlag, Berlin.

Gregg, S. J. and Sing, K. S.: 1967, *Adsorption Surface Area and Porosity*, Academic Press, New York.

Harkins, W. D. and Jura, G.: 1944, 'Vapor Adsorption Method for Determining the Area of a Solid without Assumption of a Molecular Area', *J. Am. Chem. Soc.* 66, 1366.

Heidelbaugh, N. D. and Karel, M.: 1970, 'Effect of Water Binding Agents on Catalyzed Oxidation of Methyl Linoleate', *JAOCS* 47, 539.

Heiss, R.: 1967, *Haltarbeit und Sorptionsverhalten wasserarmen Lebensmittel*, Springer-Verlag, Berlin.

Hofer, A. A. and Mohler, H.: 1962, 'Zur Aufnahmetechnik von Sorptionsisothermen und ihre A_w wendung in der Lebensmitteln Industrie', *Trav. Chim. aliment. et d'Hygiène*, 53, 274.

Hygrodynamics Tech. Bull. #4, 'Hair Hygrometry', Hygrodynamics Inc., Silver Spring, Maryland.

Hygrodynamics Tech. Bull. #5, 'Creating and Maintaining Humidities by Salt Solutions', Hygrodynamics Inc. Silver Spring, Maryland.

Kapsalis, J. G., Wolf, M., Driver, M., and Walker, J.: 1968, 'The Moisture Isotherm as a Basis for Study of Sorption and Stability Characteristics in Dehydrated Foods', *Proc. 16th Res. Conf. AMIF*, Chicago.

Karel, M. and Nickerson, J. T. R.: 1964, 'Effects of Relative Humidity, Air and Vacuum on Browning of Dehydrated Orange Juice', *Food Tech.* 18, 104.

Kuhn, I.: 1964, 'A New Theoretical Analysis of Adsorption Phenomena', *J. Colloid Sci.* 19, 685.

Labuza, T. P.: 1968, 'Sorption Phenomena in Foods', *Food Tech.* 22, 15.

Labuza, T. P.: 1971, 'Properties of Water as Related to the Keeping Quality of Foods', in *Proc. 3rd Int. Cong. Food Sci. and Tech.* p. 618.

Labuza, T. P. and Rutman, M.: 1968, 'Effect of Surface Active Agents on Sorption Isotherms of a Model System', *Can. J. Chem. Eng.* 46, 364.

Labuza, T. P. and Simon, I.: 1969, 'Surface Tension of Food Juices', *Food Tech.* 23, 96.

Labuza, T. P., Tsuyuki, H. and Karel, M.: 1969, 'Kinetics of Lipid Oxidation in Model Systems', *JAOCS* 46, 409.

Labuza T. P., Tannenbaum, S. R., and Karel, M.: 1970, 'Water Content and Stability of Low Moisture and Intermediate Moisture Foods', *Food Tech.* 24, 543.

Labuza, T. P., McNally, L., Gallagher, D., Hawkes, J., and Hurtado, F.: 1972a, 'Stability of Intermediate Moisture Foods', *J. Food Sci.* 37, 154.

Labuza, T. P., Mizrahi, S., and Karel, M.: 1972b, 'Mathematical Models for Optimization of Flexible Film Packaging of Foods for Storage', *Trans. A.S.A.E.* 15, 150.

Leistner, L.: 1970, *Arch. Lebensmittelhyg.* 21, 121 and 264.

Lajolla, F., Labuza, T. P., and Tannenbaum, S. R.: 1971, 'Reactions at Limited Water Concentration, 2. Chlorophyll Degradation', *J. Food Sci.* 36, 850.

Ling, G.: 1965, 'The Physical State of Water in the Living Cell and Model Systems', *Ann. New York Acad. Sci.* 125, 401.

Loncin, M., Bimbenet, J., and Lenges, J.: 1968, 'Influence of Water Activity on the Spoilage of Foods', *J. Food Tech.* 3, 131.

McLaren, A. D. and Rowen, J. W.: 1952, 'Sorption of Water Vapor by Proteins and Polymers: a Review', *J. Polymer Sci.* 7, 289.

Mizrahi, S., Labuza, T. P., and Karel, M.: 1970a, 'Computer Aided Prediction of Food Storage Stability: Extent of Browning in Dehydrated Cabbage', *J. Food Sci.* 35, 799.

Mizrahi, S., Labuza, T. P., and Karel, M.: 1970b, 'Feasibility of Accelerated Storage Tests for Browning of Cabbage', *J. Food Sci.* 35, 804.

Murrel, W. G. and Scott, W. J.: 1966, 'The Heat Resistence of Bacteria Spores at Various Water Activities', *J. Gen. Micro.* 43, 411.

Nemethy, G.: 1968, 'The Structures of Water and Aqueous Solutions in *Low Temperature Biology of Foodstuffs*', *Rec. Adv. Food Sci.* 4, 1.

Quast, D. G. and Karel, M.: 1972, 'Computer Simulation of Storage Life of Foods Undergoing Spoilage by two Interacting Mechanisms', *J. Food Sci.* 37, 679.

Quast, D. G., Karel, M., and Rand, W. M.: 1972, 'Development of Mathematical Model for Oxidation of Chips as a Function of Oxygen Pressure, Extent of Oxidation and Equilibrium Relative Humidity', *J. Food Sci.* 37, 673.

Oswin, C. R.: 1946, 'The Kinetics of Package Life. III: Isotherm', *J. Chem. Ind. London* 64, 419.

Richardson, G. M. and Malthus, R. S.: 1955, 'Salts for Static Control of Humidity at Relatively Low Levels', *J. Appl. Chem.* 5, 557.

Rockland, L. B.: 1957, 'A New Treatment of Hygroscopic Equilibric', *Food Res.* 2, 604.

Rockland, L. B.: 1960, 'Saturated Salt Solutions for Static Control of Relative Humidity between 5 and 40 °C. *Anal. Chem.* 32, 1375.

Salas, F. and Labuza, T. P.: 1968, 'Surface Active Agents. Effects on Drying Characteristics of Model Food Systems', *Food Tech.* 22, 1576.

Salwin, H.: 1959, 'Defining Minimum Moisture Contents for Dehydrated Foods', *Food Tech.* 13, 594.

Salwin, H. and Slawson, V.: 1959, 'Moisture Transfer in Combinations of Dehydrated Foods', *Food Tech.* 13, 715.

Schmidhofer, T. and Eoli, H.-R.: 1972, 'Zur Wasseraktivität von Fleischwaren', *Alimenta* 5, 169.

Schobell, T., Tannenbaum, S. R., and Labuza, T. P.: 1969, 'Reaction at Limited Water Concentration. 1: Sucrose Hydrolysis', *J. Food Sci.* 34, 324.

Scott, W. J.: 1957, 'Water Relations of Food Spoilage Microorganisms', in *Adv. Food Res.*, vol. III, Academic Press, New York.

Shereshefsky, J. L. and Carter, C. D.: 1950. *J. Am. Chem. Soc.* 72, 3682.

Simon, I. B., Labuza, T. P., and Karel, M.: 'Computer Aided Prediction of Food Storage Stability: Oxidation', *J. Food Sci.* 36, 280.

Stitt, F.: 1958, 'Moisture Equilibrium and the Determination of Water Content of Dehydrated Foods', in *Fundamental Aspects of the Dehydration of Foodstuffs*, Soc. Chem. Ind., London.

Strong, D. H., Foster, E., and Duncan, C.: 1970, 'Influence of A_W on the Growth of *Clostridium perfringens*, *Appl. Micro.* 19, 980.

Sudhakar, S., Steinberg, M. P., and Nelson, A. I.: 1970, 'Bound Water Defined and Determined at Constant Temperature by Wide Line NMR', *J. Food Sci.* 35, 612.

Taylor, A. A.: 1961, 'Determination of Moisture Equilibrium in Dehydrated Foods', *Food Tech.* 15, 536.

Wink, W. A. and Sears, G. R.: 1950, 'Equilibrium Relative Humidities above Saturated Salt Solutions at Various Temperatures', *Tappi.* 33, 96A.

VanArsdel, W. B.: 1963, *Food Dehydration*, Avi Pub. Co. Westport, Conn.

Zsigmondy, R.: 1911, 'Über die Struktur der Gelsder Kieselsaver Theorie der Entwässerüng', *Z. Anorg. Chem.* 71, 356.

II. Microbial Stability of IMF - Challenge Studies with Microbial Inhibitors

A. Introduction

The microbial challenge studies of Phase II, Contract NAS 9-12560 produced some findings worthy of further pursuit. The model intermediate moisture foods, Hennican, was prepared with some commonly used microbial inhibitors and humectants which were added at levels within FDA restrictions. When challenged with *Aspergillus niger*, many of the test inhibitor systems proved to be as effective as the potassium sorbate—propylene glycol system which is presently used in most IMF. At the normal a_w of the food, 0.85, *A. niger* was representative of a natural mold contaminant which could grow on that IMF product rendering it unacceptable. A product made to a higher a_w, e.g. 0.90, could support the growth of yeast and bacterial contaminants. The threat of food spoilage and food poisoning from these microbes may require that foods prepared at these high a_w's contain higher levels of microbial inhibitors or different kinds of inhibitors in order to make a microbially stable food product. In addition, a different food may require a different combination of inhibitors to be effective.

Phase III of NAS 9-12560 was centered on these aspects; (1) effectiveness of inhibitors against *Staphylococcus aureus* and (2) challenge studies in another IM product - namely semi-moist pet food. This report contains the papers presented for publication based on the results in these areas.

B. *Staphylococcus aureus* Challenge Study in an IMF

Reprinted on the next pages is a copy of the article which has been submitted to the *Journal of Food Science*. The paper was presented at the 35th Annual IFT Meeting.

Staphylococcus aureus Challenge Study in an

Intermediate Moisture Food

by

S. L. Boylan[1], K. A. Acott[2], and T. P. Labuza

Department of Food Science and Nutrition

University of Minnesota

St. Paul, Minnesota 55108

[1]Present address: Green Giant Co., LeSueur, Minnesota

[2]Present address: University of Oregon, Medical School, Portland, Oregon

1. Abstract

The effectiveness of several mold inhibitors in preventing the growth of Staphylococcus aureus in an intermediate moisture food was tested. S. aureus was inoculated into the food at a_w's of 0.86 to 0.90 and pH 5.2 and 5.6 conditions under which growth of 2-5 log cycles occurs in 2 weeks. Not all inhibitors prevented growth. The effectiveness against S. aureus was a function of both pH and a_w. Of the compounds tested, methyl paraben, sodium benzoate, potassium sorbate and calcium propionate proved to be the most effective against S. aureus at low concentrations.

2. Introduction

Intermediate moisture foods (IMF) have received widespread attention in recent years. The popularity of convenience foods, which can be stored at room temperature and which require no further preparation before consumption, has caused many manufacturers to develop intermediate moisture formulations for human and pet foods (Bone et al., 1974). The relatively low moisture contents which characterize IMF can result in the production of foods which are microbiologically shelf stable under conditions of room temperature storage. As the moisture content or water activity (a_w) of a food is lowered, the amount of water available for supporting microbial growth is lessened (Troller, 1973; Scott, 1957). Generally, yeasts and molds are able to grow in environments with lower a_w's than are bacteria. The pathogen, Staphylococcus aureus, is unusual in that it can grow down to a_w 0.84-0.85 and produce toxin, as reviewed by Troller (1973).

Previous work in our laboratories tested the effectiveness of several common food additives at a_w 0.85 in preventing the growth of the mold, Aspergillus niger, in a model IMF (Acott and Labuza, 1975). This a_w permits the growth of many molds but inhibits the growth of almost all bacteria, especially the growth of any potentially pathogenic bacteria. However, in the formulation of IMF, it is often desirable to have an a_w of somewhat greater than 0.85 to create an acceptable product from a textural standpoint. As stated, the growth of Staphylococcus aureus can begin at an a_w of about 0.84-0.86, depending on the product (Tatini, 1973; Leistner, 1970). During growth, S. aureus produces enterotoxins which can cause food intoxication and thus pose a potential health hazard even at these lower a_w's.

The release of enterotoxins by S. aureus begins during the early log phase of growth when the population reaches about 10^6 CFU/gram (Tatini, 1973). However, if conditions are correct, enterotoxin may be produced by high populations of nongrowing cells (Martus and Silverman, 1968, 1969). As reviewed by Minor and Marth (1972), staphylococcal contamination is very common in a wide variety of foods. If an IMF food became contaminated with S. aureus, enterotoxin production could occur if there was considerable S. aureus growth.

In an attempt to prevent enterotoxin production by S. aureus in IMF, it would be desirable to determine which chemical compounds used as microbial inhibitors in foods prevent staphylococcal growth. If S. aureus growth were inhibited, the chance that enterotoxin would be produced in IMF would be lessened. The present study was designed to determine the effects of the previously tested mold inhibitors on the growth of S. aureus F265. Enterotoxin production was not measured in this study. The model IMF food was formulated to the a_w range of 0.86-0.90 which would support the growth of staphylococci.

Because of physiological differences, molds, yeasts, and bacteria may be affected differently by microbial inhibiting compounds. Some inhibitors are very effective against molds and yeasts but are much less inhibitory to bacteria. Thus, one could not assume that a compound which proved to be effective in inhibiting A. niger would prevent the growth of S. aureus F265.

The pH of a food system also may influence the effectiveness of a microbial inhibitor. The acid type inhibitors must be in the undissociated form to be inhibitory to microbes (Sauer, 1972 and Ingram et al., 1956). As the pH of the food to which the inhibitor has been added is lowered, the proportion of the inhibitor in the undissociated state increases and increases its effectiveness. Chichester and Tanner (1968) suggest that this is because the undissociated form is highly lipid soluble and accumulates in the lipid structures of the cells inhibiting cell metabolism.

-50-

Parabens and polyhydric alcohols were also studied. The parabens are very effective against many microbes, especially mold and Gram positive bacteria (Furia, 1968). The parabens are more effective at a high pH than are the acid-type inhibitors. Methyl and propyl paraben were used together in one test system because the best inhibitory effect is often obtained by using a combination of the two compounds (Chichester and Tanner, 1968). The polyhydric alcohols bind water and inhibit microbial growth by lowering the a_w of the food system. These compounds may inhibit microbial growth in other ways in addition to lowering the a_w.

Caproic acid, a long-chain fatty acid, has been suggested for use instead of potassium sorbate in intermediate moisture dog foods (Haas, 1973). This compound has not been approved by the FDA for use in foods.

3. Materials and Methods

Model IMF Formulation

The composition of the model IMF food (Hennican) is shown in Table 1. The natural pH of this system was 5.6. The unsalted, hulled peanuts (Skippy Co., Minneapolis, MN) were ground in a blender. The chicken (Aslesen's Banquet Table, canned deluxe boned) was freeze-dried and ground in a silent cutter. Nonfat dry milk, ground peanuts, and chicken were mixed in a 500 ml Brabender-Farinograph bowl.

Raisins were ground in a food grinder and blanched in a microwave oven for 1.5 min to destroy active enzymes. The raisins, peanut butter (Skippy, creamy style), and honey were added to the dry ingredients in the BrabenderFarinograph bowl as mixing continued. The water was added slowly and the IMF was mixed at high speed for 5 min.

Pimaricin, an antimycotic agent, was added because initial work showed that the Hennican contained large amounts of a natural mold contaminant. The growth of this mold inhibited staphylococcal growth in

Table 1. Hennican Formulation

Ground Peanuts	13.37 grams
Ground, freeze-dried chicken	13.37 g
Non-fat, dry milk	9.73 g
Ground raisins	26.73 g
Peanut butter	3.53 g
Honey	1.44 g
Water	33.00 g
Pimaricin	0.002 g

$A_w = 0.91$

$pH = 5.6$

the model IMF. In order to eliminate this, pimaricin was used. This compound has been found to be effective in inhibiting over 500 fungi but has no effect on the growth of bacteria or viruses (Clark et al., 1964). To verify that pimaricin would have no effect on staphylococcal growth in our system, it was added to the model IMF at a level of 0.002%. As shown in Figure 1, in the samples to which no pimaricin was added, considerable mold growth occurred after 7-10 days and staphylococcal growth was inhibited. Growth of the S. aureus F265 continued in the Hennican samples to which pimaricin had been added and no mold was observed. This showed that pimaricin could inhibit mold growth in Hennican without inhibiting staphylococcal growth. Pimaricin was added to the Hennican by placing it in the water before the water was mixed into the product.

Addition of Inhibitory Compounds

The first part of the study was done by adding the inhibitory compounds to the model system at pH 5.6. In the second part, the system was acidified to pH 5.2 using citric acid so the effect of slightly lowered pH on the staphylococci could be measured. The pH was measured directly with a Beckman nonaqueous electrode. A pH of 5.2 was chosen because preliminary work showed that staphylococcal growth was best in our system at or above this pH, and at lower pH the food was organoleptically unacceptable. In a study by Scheusner and Hanson (1973), it was shown that no enterotoxin was detectable in foods below pH 5.0; however, Tatini (1972) showed growth and enterotoxin production at pH 4-4.5. Thus, a pH of 5.2 should not prevent enterotoxin production by growing staphylococci. Since it was believed that a lowered pH would result in increased bacterial inhibition, lower levels of the inhibitors than those which proved to be effective at pH 5.6 were tested in the systems at pH 5.2.

FIGURE 1

EFFECT OF PIMARICIN ON S. AUREUS AND MOLD GROWTH
IN AN INTERMEDIATE MOISTURE FOOD

S. aureus in Hennican,
a_W 0.90, pH 5.6

PIMARICIN
0.002 %

NO
PIMARICIN
(Control)

Mold appeared
on control

LOG VIABLE FRACTION, N/N_0

TIME (days)
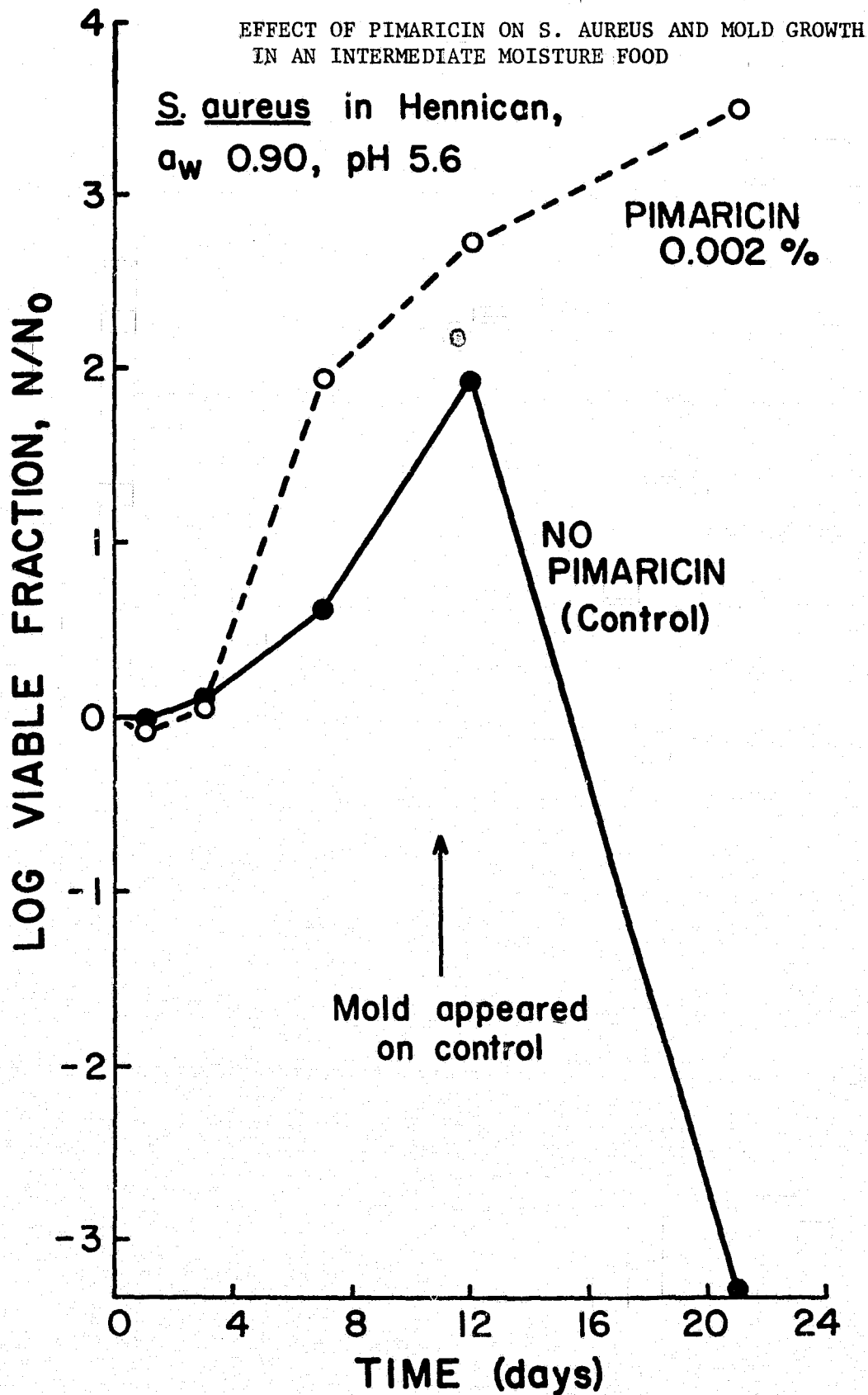
The inhibitors tested in this study are shown in Table 2. The acid type and paraben inhibitors were added at the desired levels to 50 gm of Hennican. It was found that these additives did not affect the a_w of the product. The polyhydric alcohols were added to 150 gm portions of Hennican in the desired amounts. The amount of each humectant required to lower the food to a_w's 0.86 and 0.88 had been determined previously experimentally. Unfortunately, the heterogeneity of the Hennican system made it very difficult to obtain exactly the desired a_w repeatedly with addition of the humectants. From each 150 gm system, 100 gm were used to measure the a_w and 50 gm were used for inoculation with S. aureus. The Fett-Vos isopiestic method for a_w determination was used in this study (Vos & Labuza, 1974). The addition of the inhibitors did not markedly affect the pH of the Hennican systems.

Inoculation with S. aureus F265

Each 50 gm portion was inoculated with a Staphylococcus aureus F265 culture which had been grown in trypticase soy yeast extract broth at 23°C for 24 hr. The culture was diluted to obtain the desired population for inoculation of the test systems. The level of inoculation was approximately 3×10^5 colony forming units per gm of food. After thorough mixing, each system was divided into ten 5 gm samples which were placed in 60x15 mm plastic petri dishes. The samples were stored in desiccators over saturated salt solutions.

Conditions of Storage

Storage within a desiccator over the appropriate saturated salt solution maintains the proper a_w of the samples by preventing them from drying out. The saturated salt solutions used in the desiccators for storage were: $BaCl_2$ (a_w=0.90), $ZnSO_4$ (0.88), and KCl (0.86). Samples to which no inhibitors (controls), acid-type inhibitors, or parabens

Table 2. Test Systems for the Inhibition Study

Inhibitor	pH 5.6		pH 5.2	
	% level (w/w)	Storage A_w	% level (w/w)	Storage A_w
Methyl paraben	0.2*	0.90	0.07	0.90
	0.4*	0.90	0.10	0.90
Propyl paraben	0.03	0.90	0.03	0.90
	0.05	0.90	0.05	0.90
Methyl Propyl paraben	0.02	0.90	0.02	0.90
	0.05	0.90	0.05	0.90
Sodium benzoate	0.05	0.90	0.20	0.90
	0.10	0.90	0.30	0.90
Calcium propionate	0.20	0.90	0.05	0.90
	0.30	0.90	0.10	0.90
Potassium sorbate	0.10	0.90	0.10	0.90
	0.30	0.90	0.30	0.90
Caproic Acid*	Not tested		0.10	0.90
			0.30	0.90
Propylene Glycol	7.0	0.88	1.0	0.88
	10.8	0.86	3.0	0.88
1,3 Butylene Glycol*	5.0	0.88	1.0	0.88
	10.0	0.86	3.0	0.88
Glycerol	1.5	0.88	1.5	0.88
	6.0	0.86	6.0	0.88
Polyethylene glycol 600	3.0	0.88	1.0	0.88
	10.0	0.86	3.0	0.88
Sorbitol	3.0	0.88	3.0	0.88
	10.0	0.86	8.0	0.88
Control	0	0.90	0	0.90

*Not approved by FDA or above FDA level

had been added were stored at a$_w$ 0.90. Samples to which low levels of polyhydric alcohols were added were stored at 0.88 and those with higher levels of these compounds were stored at 0.86, as shown in Table 2. Samples were stored at 22°C.

Enumeration of the S. aureus F265

Five g samples were combined with 45 gm of sterile phosphate buffer and blended for surface plating on tryticase soy agar to which 5 gm of yeast extract per liter had been added. The plates were incubated at 37°C and colonies were counted after 48 hr. The samples were plated for enumeration of the S. aureus immediately after inoculation and at various time intervals to monitor the bacterial population changes.

4. Results and Discussion

In monitoring the staphylococcal population changes throughout the storage periods, several types of growth patterns were observed. Figure 2 shows a graph of population changes in some representative Hennican systems at pH 5.2.

As can be seen from Figure 2, there were some systems in which the staphylococcal populations increased rapidly during storage. In the non-acidified control at pH 5.6. S. aureus growth was rapid. The control system at pH 5.2 showed slower yet still noticeable growth. When glycerol (1.5%) was added to a pH 5.2 system, growth was more rapid than without its addition yet slower than in the pH 5.6 control system. Glycerol probably acted as an energy source for the staphylococci (Patsch & Hoehne, 1967).

In the methyl paraben (0.10%) system, a bacteriostatic response is illustrated. The population level changed very little during 100 days of storage. Since staphylococcal growth did not occur in this system, methyl paraben was assumed to be an effective inhibitor of staphylococcal growth.

FIGURE 2

EFFECTIVENESS OF VARIOUS CONDITIONS AND ADDITIVES ON
S. AUREUS IN AN INTERMEDIATE MOISTURE FOOD AT A_W 0.9
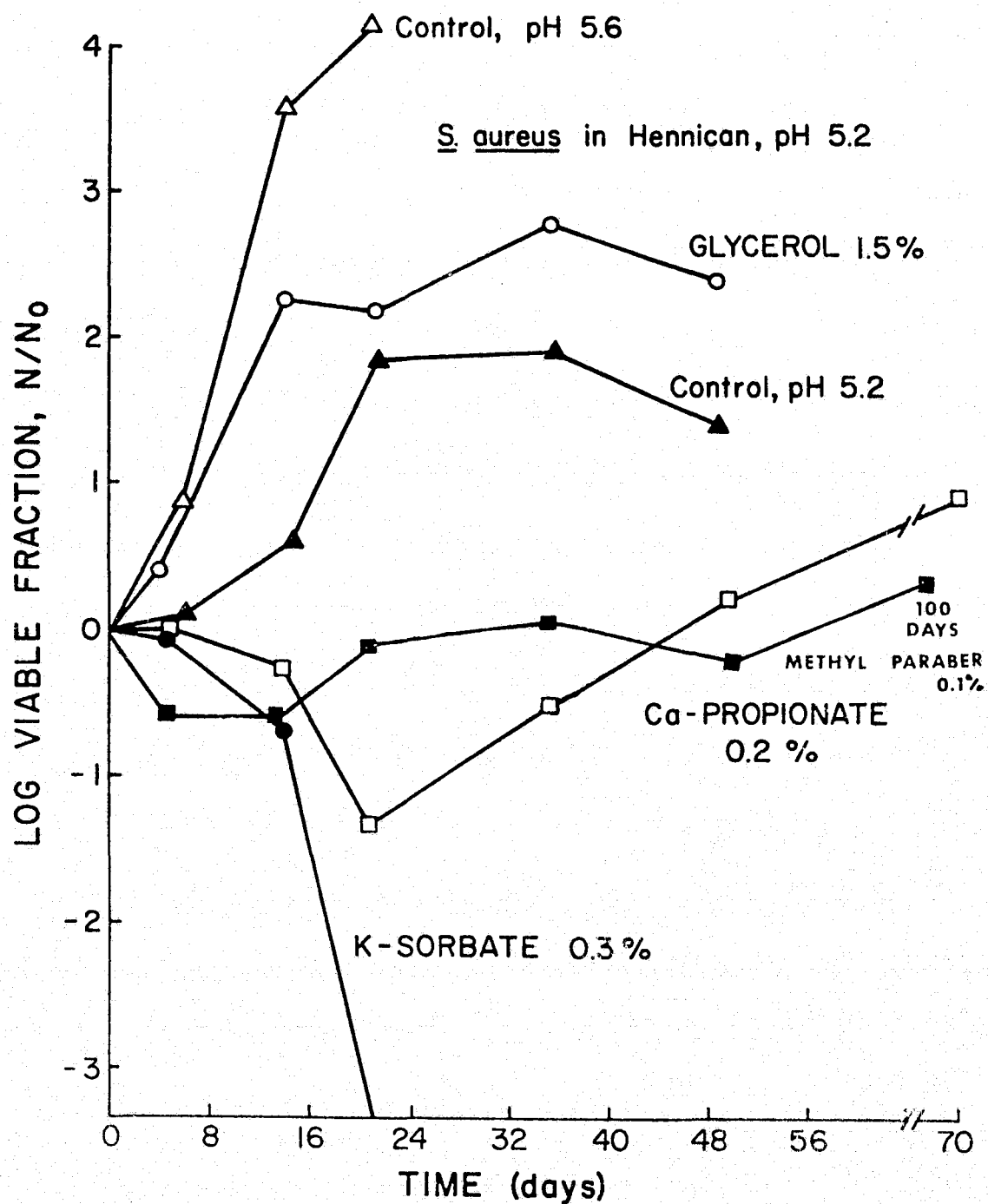
In the potassium sorbate (0.30%) system, the bacterial population decreased continuously. This compound proved to be an effective inhibitor and inactivator of staphylococci at this level of addition.

Another growth trend is illustrated by the calcium propionate (0.20%) system. As shown, the population decreased within the first three weeks of storage but then began to increase steadily. This delayed increase in staphylococcal growth may have been due to the eventual metabolic reduction of the inhibitory propionate which decreased the compound's inhibiting effects. If population monitoring had been terminated within the first few weeks of storage when the population levels appeared to be decreasing, the later increases would have been undetected. This illustrates the vital importance of extended storage periods in challenge studies. In our work, platings to monitor staphylococcal growth were continued in all cases until a definite growth pattern was established. The growth patterns shown in Figure 2 are from systems at pH 5.2 but similar types of growth trends were shown at pH 5.6.

Table 3 shows the levels of the compounds which proved to be effective for inhibiting staphylococcal growth in Hennican at the two pH's tested. As expected, decreasing the pH of the model system increased the effectiveness of several of the inhibitors. In the case of the acid type inhbitors, decreasing the pH of the test system decreased the dissociation of these compounds, enhancing their inhibitory effects, as described previously. In the other systems, it is likely that decreasing the pH caused increased stress for the staphylococci. S. aureus grows best near neutrality. Although there may not have been a synergistic effect between the increasing acidity and these inhibitory compounds, an additive effect between the acidity and the inhibitor resulted in greater microbial inhibition at the lower pH.

TABLE 3

LEVELS OF COMPOUNDS EFFECTIVE IN PREVENTING STAPHYLOCOCCAL GROWTH AT HIGH A_W

	pH 5.6	
Inhibitor	% Level (w/w)	A_w
Methyl paraben	0.4	0.90
Propylene glycol	7.0	0.88
1,3 Butylene glycol	5.0	0.88
Polyethylene glycol 600	10.0	0.88

	pH 5.2	
Methyl paraben	0.1	0.90
Sodium benzoate	0.1	0.90
Potassium sorbate	0.1	0.90
Calcium propionate	0.3	0.90
Caproic acid*	0.1	0.90
1,3 Butylene glycol	3.0	0.88

The polyhydric alcohols used to lower the a_w of the systems varied in their effectiveness. Propylene glycol and polyethylene glycol 600 were effective at high levels of addition but did not prove effective at lower levels even with the additional stress of lowered pH. Neither glycerol nor sorbitol proved inhibitory even at high levels. It seems likely that these compounds were metabolized by the staphylococci. When compared to the other polyhydric alcohols, 1,3 butylene glycol was found to be an effective inhibitor at the lowest level of addition. At the present time, this compound is not approved by the FDA for use in foods as a microbic inhibitor. The results of this study indicate that 1,3 butylene glycol appears to be an effective inhibitor of <u>S. aureus</u> F265 and that it should be investigated further in an attempt to get FDA approval for its usage as a microbial inhibitor.

Methyl paraben proved to be more effective than propyl paraben at the levels of addition employed. The combination of methyl and propyl parabens did not prove to be effective. It should be noted that methyl paraben was added at a higher level than was propyl paraben or the combination of the two parabens so this may explain the differences observed.

At pH 5.2, caproic acid was found to be a very effective inhibitor of <u>S. aureus</u> F265. This indicates that this compound is worthy of further study for use as a microbial inhibitor in IMF.

This study has shown that <u>**S. aureus**</u> F265 growth can occur at a_w's near 0.90 and can pose a potential health hazard in intermediate moisture foods. The compounds tested in this study were found to be less effective in inhibiting <u>S. aureus</u> at a_w's ranging from 0.86 to 0.90 than they were in inhibiting <u>A. niger</u> at a_w 0.85. Lowering the pH of the model IMF system by the addition of citric acid increases the effectiveness of several of the inhibitors, most noticeably the acid-type compounds which are most effective in the undissociated forms found in the greatest amounts at low pH values.

5. References

Acott, K.M. and T.P. Labuza. 1975. Inhibition of Aspergillus niger in an intermediate moisture food system. J. Food Sci. 40: 137.

Bone, D.P., Ross, K.D., Shannon, E.L. and Flanyak, J.R. 1972. Some factors in formulating and processing intermediate moisture foods. Proc. AACC Meeting, October. Montreal, Canada.

Chichester, D.F. and Tanner, F.W., Jr. 1968. Antimicrobial food additives. Handbook of Food Additives. T.E. Furia, editor. Chemical Rubber Co., Cleveland, Ohio.

Clark, W.L., Shirk, R.J. and Kline, E.F. 1964. Pimaricin, a new fungistat. 4th Int'l Symposium on Food Microbiology. SIK. Goteborg, Sweden.

Frankenfeld, A.J.W., Karel, M. and Labuza, T.P. 1973. Intermediate moisture food composition containing aliphatic 1,3-diols. Patented May 8, 1973. No. 3,732,112.

Furia, T.E., editor. 1968. Handbook of Food Additives. Chemical Rubber Co., Cleveland, Ohio.

Haas, G.J. 1973. Canadian Patent #922961. Animal Food Products. Granted to General Foods Corporation, White Plains, New York.

Ingram, M., Ottaway, F.J.H. and Coppock, J.B.M. 1956. The preservative action of acid substances in food. Chem. & Ind. Part 2. pp. 1154-1163.

Leistner, L. 1970. Arch. Lebensmittel Hyg. 21: 121.

Minor, T.E. and Marth, E.H. 1972. Staphylococcus aureus and staphylococcal food intoxications: A review. IV. Staphylococci in meat, bakery products and other foods. J. Milk Food Technol. 35: 228.

Patsch, R. and Hoehne, C. 1967. Investigation of the influence of solvents on bacteria growth and their application in the determination of bacteria resistance to chemotherapeutics hardly soluble in water. Arch. Hyg. 151: 91.

Plitman, M., Park, Y., Gomez, R. and Sinskey, A.J. 1973. Viability of Staphylococcus aureus in intermediate moisture meats. J. Food Sci. 38: 1004.

Sauro, F. 1972. Control of mold by chemical preservatives. Fungi and Food. 7th Annual Symposium, Western New York Section, IFT. October 19, 1972.

Scheusner, D.L. and Harmon, L.G. 1973. Growth and enterotoxin production by various strains of Staphylococcus aureus in selected foods. J. Food Sci. 38: 474.

Schmidt, E.W., Jr., Gould, W.A. and Weiser, H.H. 1969. Chemical preservatives to inhibit the growth of S. aureus in synthetic cream pies adjusted to pH 4.5—5.0. Food Technol. 23: 1197.

Scott, W.J. 1957. Water relations of food spoilage microorganisms. Adv. Food Res. 7: 83.

Tatini, S.R., Jezeski, J.J., Olson, J.C., Jr., and Casman, C.R. 1971. Factors influencing the production of staphylococcal enterotoxin A in milk. J. Dairy Sci. 54: 312.

Tatini, S.R. 1973. Influence of food environments on growth of Staphylococcus aureus and production of various enterotoxins. J. Milk Food Technol. 36: 559.

Troller, J.A. 1973. The water relations of food-borne bacterial pathogens: A review. J. Milk Food Technol. 36: 276.

Vos, P.T. and Labuza, T.P. 1974. Technique for measurement of water activity in the high a_w range. J. Agric. Food Chem. 22: 326.

6. Additional Results

Although pimaricin seemed to be effective in delaying
mold growth, it didn't prevent mold growth on all the samples throughout
the storage study as seen in Table 4. The previous results showed
that S. aureus growth, however, was not inhibited by 0.002% of pimaricin
but actually seemed to enhance S. aureus growth in some cases. Perhaps
pimaricin was metabolized by S. aureus. In the systems where significant
S. aureus growth had occurred, the pimaricin may have been removed
from the systems during the bacterial metabolism. When the pimaricin
was removed, mold growth could begin. It was found, however, that when
mold growth became profuse, staphylococcal growth was inhibited. As
seen in Figure 3, after mold growth appeared, the staphylococcal
population began to decline. The bell-shaped curve reflects three
possible mechanisms:

a. The growth of staphylococci and breakdown of pimaricin.

b. Disappearance of pimaricin, the growth of mold and

the inhibition of staphylococci.

c. Inhibition of staphylococci by its own metabolic

products, i.e. end of stationary phase and entering

death phase.

Note from Table 4 that systems where mold growth did occur,
calcium propionate, K-sorbate, propylene glycol, 1,3, butylene glycol,
were systems which have previously been shown to be especially antimycotic
(Phase ii, Plitman, 1973). Propylene glycol and 1,3 butylene glycol
are also antibacterial as seen in Figure 4 so the inhibition of the mold
was due to the presence of pimaricin and the polyol. Calcium propionate

TABLE 4

TIME OF MOLD APPEARANCE ON HENNICAN (pH 5.6)

Inhibitor	% Level (w/w)	Day of Appearance
Methyl paraben	0.2	19
	0.4	none*
Propyl paraben	0.03	19
	0.05	19
Methyl/propyl paraben	0.02	19
	0.05	19
Sodium benzoate	0.05	19
	0.10	27
Calcium propionate	0.20	none*
	0.30	none*
Potassium sorbate	0.10	none*
	0.30	none*
Propylene glycol	7.0	none*
	10.0	none*
1,3 Butylene glycol	5.0	none*
	10.0	none*
Glycerol	1.5	32
	6.0	39
Polyethylene glycol 600	3.0	32
	10.0	33
Sorbitol	4.3	32
	14.3	51
Control	0	19

* No mold growth by day 51

FIGURE 3

S. aureus GROWTH IN HENNICAN
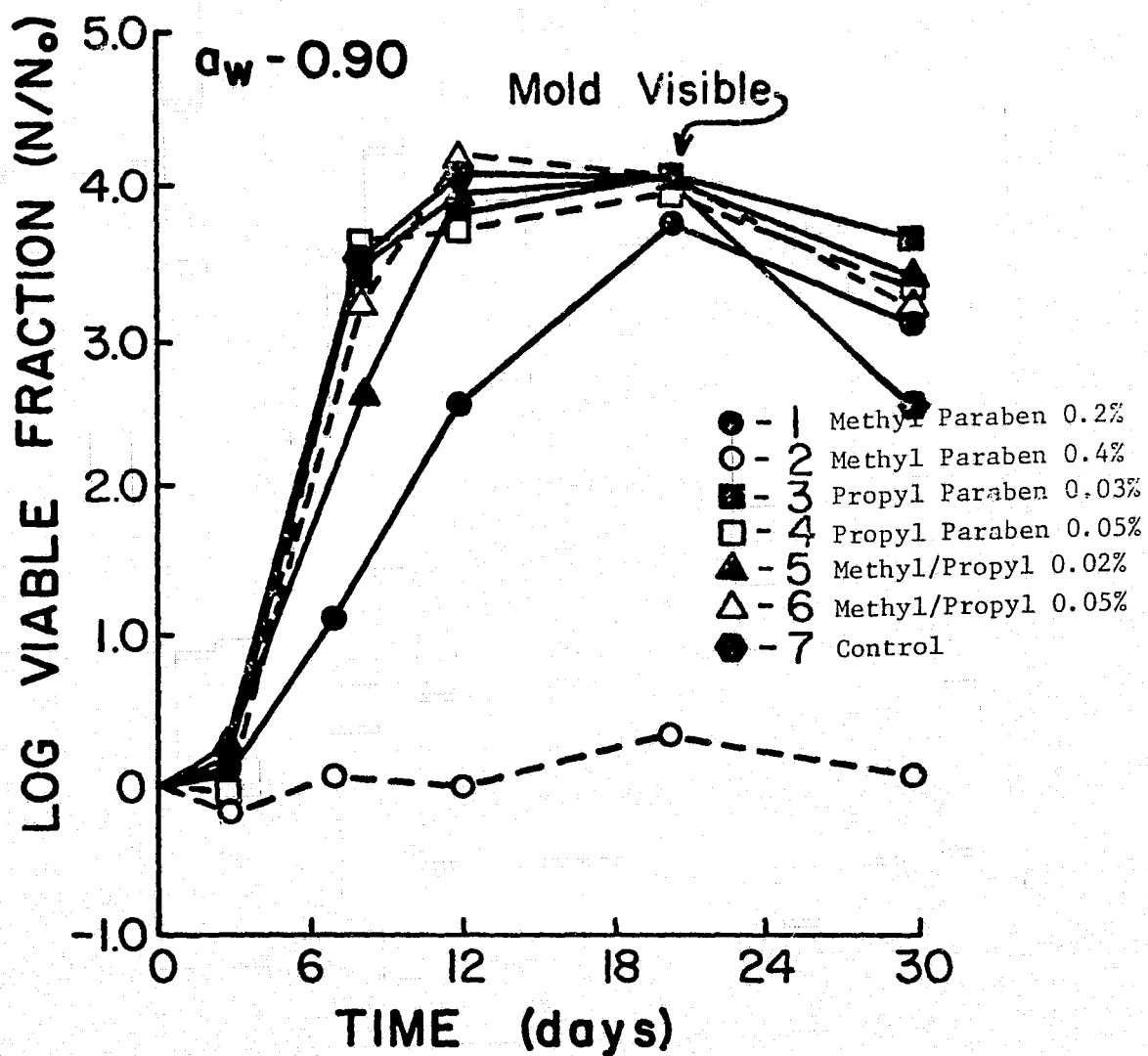
FIGURE 4

S. aureus GROWTH IN HENNICAN

Figure with Y-axis "LOG VIABLE FRACTION (N/N₀)" ranging from -4.0 to 5.0, X-axis "TIME (days)" ranging from 0 to 36.

Legend:
- ● − 1 Propylene glycol 7%
- ○ − 2 Propylene glycol 10%
- ■ − 3 1,3 Butylene glycol 5%
- □ − 4 1,3 Butylene glycol 10%
- ⬡ − 5 Control
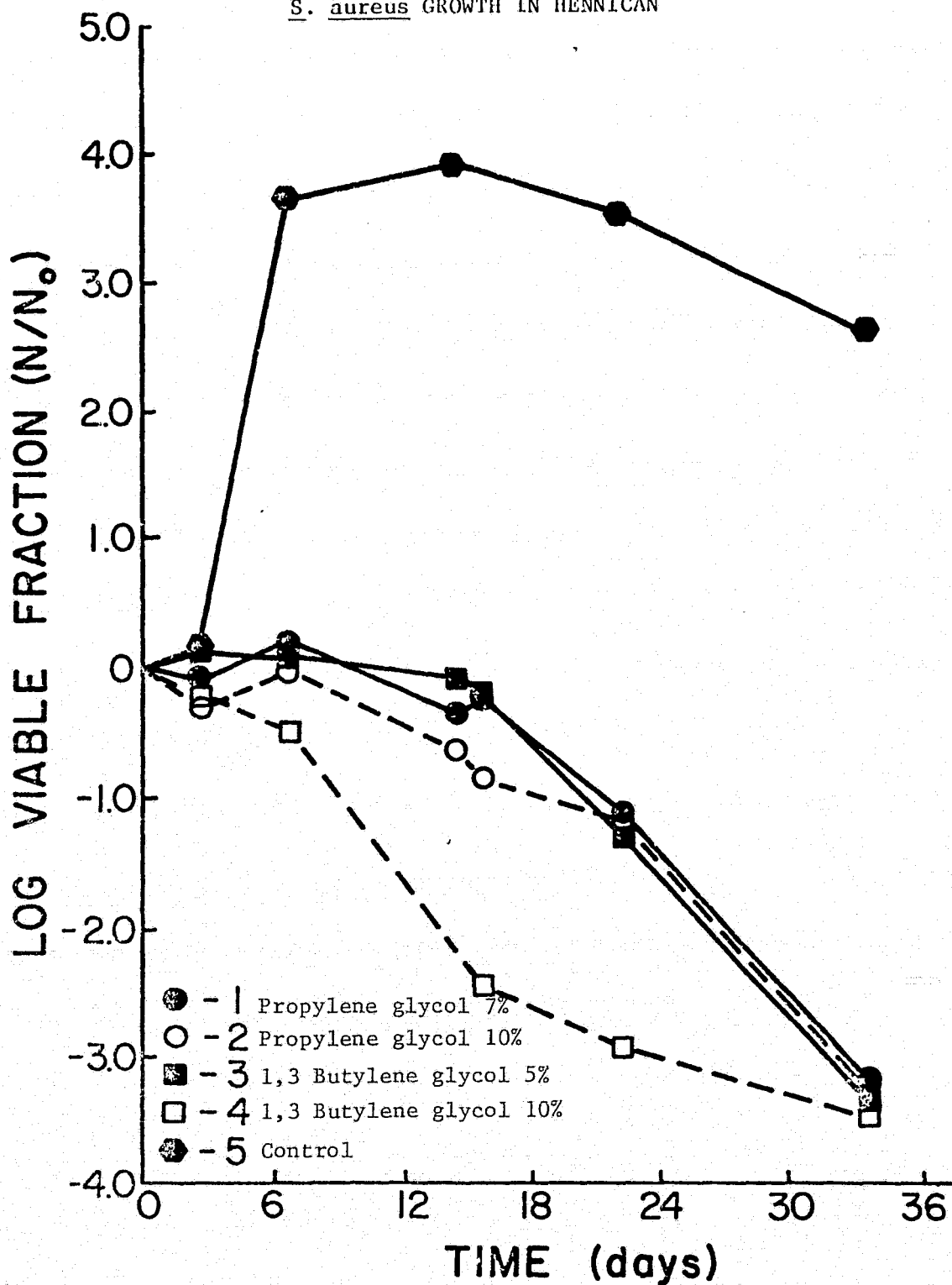
and K-sorbate didn't inhibit the staphylococci so the mold was inhibited primarily by these inhibitors since the pimaricin was probably metabolized during the staphylococcal growth in these two systems.

The 10% polyethylene glycol 600, which was bacteriostatic to the staphylococci, developed mold growth by 33 days. It is possible that although the staphylococcal count didn't increase, the viable population of staphylococci may have been metabolizing the pimaricin thus allowing mold growth. The degradation of pimaricin could have also resulted from other causes. According to a manufacturer's publication (Gist-Brocades N.V., 1972) pimaricin at the pH of these test systems should be 100% active for three weeks at 30°C if protected from ultraviolet light, heavy metal ions and oxidants. Although the food systems were not exposed to ultraviolet light, it is possible that heavy metal ions (Fe^{++} in raisins) or oxidants in the Hennican promoted the breakdown of pimaricin.

C. Effect of Pimaricin on $\underline{S}$. $\underline{aureus}$ Growth in a Liquid System

1. Introduction

In the previous section it was shown that $\underline{S}$. $\underline{aureus}$
growth was slightly promoted in a solid IMF system. This short study
was done to confirm this in a liquid medium highly suitable for
$\underline{S}$. $\underline{aureus}$ growth.

2. Methods

a. $\underline{S}$. $\underline{aureus}$ growth was measured in BHI broth. The
Brain-Heart Infusion medium was mixed according to instructions in
the Difco Manual.

b. Various amounts of glycerol were added to lower the
a_w of the sterile BHI and the a_w was measured by the VPM technique.

c. Two 250 ml flasks containing the BHI or BHI-glycerol
mixture were used for each a_w to be studied.

d. To one flask at each a_w, 0.002% (w/w) pimaricin was
added. The other was used as a control.

e. Each of the flasks was inoculated with 0.1 ml of
$\underline{S}$. $\underline{aureus}$ stock culture grown in BHI at $23^{\circ}C$ on a shaker for 18 hr
at 115 opm.

f. The cultures were plated at several intervals to
determine the staphylococcal population levels.

3. Results

The study in the BHI-glycerol liquid media where mold was
not a factor was designed to show if pimaricin could be stimulatory
to $\underline{S}$. $\underline{aureus}$ over a range of a_w's. The results appear in Figure 1.
The different growth response of the $\underline{S}$. $\underline{aureus}$ at the various a_w's
is obvious. Growth occurred above a_w 0.88 and inactivation was

FIGURE I

EFFECT OF PIMARICIN GROWTH OF STAPHYLOCOCCUS AUREUS IN
BRAIN-HEART INFUSION AS A FUNCTION OF A_W AT $35^\circ C$
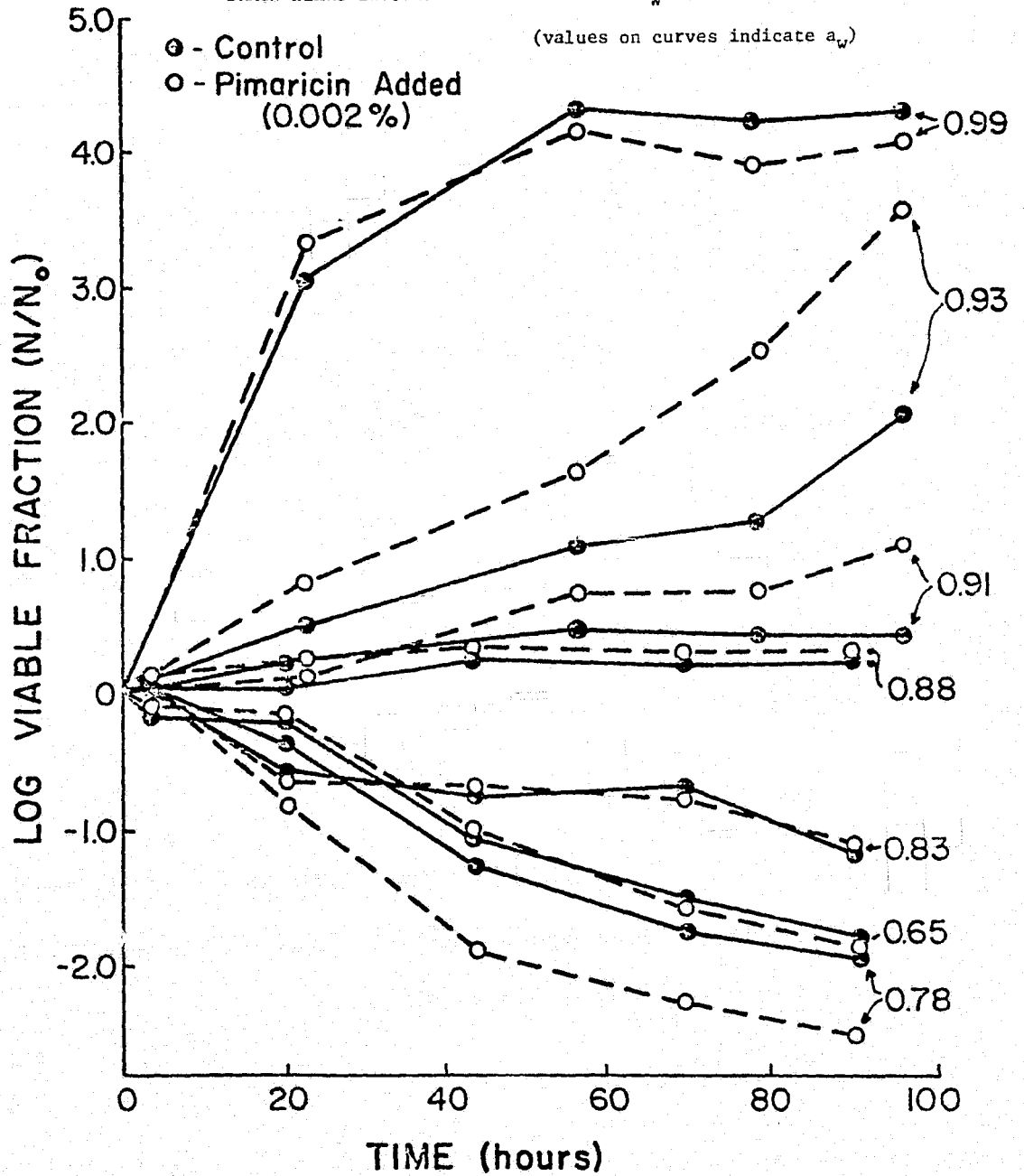
apparent at a_w 0.83 and below. At some a_w's the effect of pimaricin on the S. aureus growth was insignificant and within the error of the plating procedure. The S. aureus growing in the BHI-glycerol solutions at a_w 0.91 and 0.93 showed a 1 and 2-fold difference respectively between the system with and that without primaricin added, the S. aureus growing more rapidly with the added pimaricin. On the other hand in the a_w 0.78 system, pimaricin caused a 0.5 log cycle increase in S. aureus inactivation. Note also the reversal of inactivation rate as seen before (see Final Report, Phase I, pp. 202-204). The rate of inactivation at a_w 0.78 is faster than at a_w 0.65. The reason for this reversal is still not known. It appears here that pimaricin, although slightly stimulatory at the other a_w's, is slightly inhibitory at a_w 0.78.

The results obtained seem to show that pimaricin may slightly stimulate S. aureus growth at a_w's between 0.88 and 0.99. It is possible that the higher population of S. aureus in the Hennican plus pimaricin samples (Section II, A.) can be attributed to a stimulation of the bacteria's growth and subsequent inhibition of the mold.

It is thus felt that the antibiotic, pimaricin, can be used in the future to study the inhibition of S. aureus growth in inter-mediate moisture foods. It is desirable in a test of the inhibition of S. aureus that its growth be under optimum conditions as it would be in a food. If an inhibitor is found effective in preventing the growth of S. aureus in a non-competitive environment, that inhibitor should also be effective in a food with a water activity in the range where

mold and Staphylococcus sp. could be co-contaminants (0.88).

Staphylococcus sp. is the most important challenge to a food system

since its rate of growth is faster than molds and it has a greater

resistance to the polyols which may be included in the formulation

of the product.

    D. Microbial Challenge Studies with an Intermediate Moisture

        Dog Food

        Reprinted on the next pages is a copy of the article which

has been submitted to the Journal of Food Science. The paper was

presented at the 35th Annual Meeting of the Institute of Food

Technologists.

MICROBIAL CHALLENGE STUDIES WITH AN

INTERMEDIATE MOISTURE DOG FOOD

K. Acott, A.E. Sloan and T.P. Labuza

Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

1. Abstract

Various microbial inhibitor systems were studied for their effectiveness in a semi-moist dog food formulation. The effectiveness was measured as inhibition of the natural contaminants Aspergillus glaucus and Staphylococcus epidermitis and of an inoculated challenge organism, Aspergillus niger. The study was carried out at a_w 0.85 and 0.88 and at pH 5.4 and 6.3 giving a total of 108 systems. It was found that very few compounds alone or in combination could prevent growth of all three organisms. The only FDA approved additive that was effective alone was propylene glycol.

2. Introduction

Intermediate moisture food (IMF) products and technology are among the newest in food science. Although semi-moist foods have been thoroughly researched, as indicated by many authors the factors determining their stability have not been solved (Bone, 1973; Labuza, 1974 and Haas, 1975). These IM foods are formulated to borderline conditions with respect to microbial growth. A relatively moist product is necessary for palatability but the water activity (a_w) must be below that allowing the growth of most microorganisms, especially Staphylococcus aureus which can grow to a_w 0.84. Thus the osmotolerant bacteria, yeasts and molds are usually inhibited by antimicrobial food additives included in the product formulation.

With respect to the current IM dog foods, reformulation is inevitable due to the rising cost of various ingredients such as propylene glycol and sugar which act as antimicrobial agents. This study investigated the effectiveness of several antimicrobial agents

-74-

at two water activities. The effects were studied by a microbial

challenge procedure run in parallel with unchallenged samples for

comparison.

The microbes that occur naturally in semi-moist dog

food used were Aspergillus glaucus and Staphylococcus epidermitis

(positive identification was based on typical morphological characteristics

and biochemical tests). The activity of these microbes is due to

their ability to grow at low a_w's. $\underline{A}$. glaucus can grow at a_w 0.73-0.75

(Christensen and Meronuck, 1974). In this study, $\underline{S}$. epidermitis grew

in the dog food as low as a_w 0.82. If it is prevented from growing, one

can assume that Staphylococcus aureus will not grow (a_w minimum 0.84).

The challenge organism was Aspergillus niger. Although not as

osmotolerant as $\underline{A}$. glaucus, this mold grows at the lowest a_w studied

with the dog food. The minimum a_w for growth of $\underline{A}$. niger in a chicken

based IMF was 0.79 (Acott and Labuza, 1975). Use of these three

organisms constitutes potential growth of both the natural contaminants

and that of a ubiquitous air-borne mold that could inoculate the

product and cause loss of acceptability.

3. Materials and Methods

Preparation of test systems

The semi-moist dog food used in this study was formulated

at the Quaker Oats Co. (Barrington, IL), excluding the antimicrobial

system, phosphoric acid, propylene glycol and K-sorbate. After

extrusion and canning the dog food was held in frozen storage (-29°C)

until used. After thawing and tempering to 23°C, the dog food for

each study was aseptically taken from the cans and ground in a sanitized

Hobart Silent Cutter (Hobart Manufacturing Corp., Troy, Ohio) to
ensure homogeneity. An acidulant was added to give a pH of 5.4.
Adipic, citric, fumaric, lactic and phosphoric acid were tested but
none exhibited specific antimicrobial properties. Phosphoric acid,
the least expensive one, was used in the subsequent studies.
Figure 1 shows the scheme used to prepare the various test systems.
After acidification the batch was divided into two parts and sterile
water was added to one half giving an a_w of 0.88. The initial a_w
of the other half of the dog food was 0.85. These systems were
further divided and the inhibitors were added to give the desired
levels. Following a 24 hr equilibration period at $23^{\circ}C$, each inhibitor
system was divided into two equal parts (25 grams each). Sterile water
(0.1 ml) was added to one and the other was inoculated with an
A. niger spore suspension (0.1 ml). The water and suspension were mixed
into the systems by blending in an Osterizer blender jar (1 cup size)
for 1 minute, stopping 2-3 times to scrape the material into the blades.
The initial population of A. niger was 10^6 colony forming units (CFU)/
100 grams of dog food. The inoculation procedure did not cause a change
in the a_w of the systems. The 5 gram samples were weighed into petri
dishes (60 x 15 mm) and stored in closed chambers over saturated salt
slurries of the appropriate a_w, Li_2SO_4 = 0.85 and $ZnSO_4$ = 0.88. The
chamber lids were removed periodically to ensure adequate aerobiosis.
The slurries were stirred as necessary for maintenance of the proper a_w.

Antimicrobial agents

The antimicrobial agents which were tested alone or in
combination were of three types: metabolic inhibitors, polyhydric
alcohols (polyols), and sugars. Each chemical and the FDA-approved

FIGURE 1

FLOW DIAGRAM FOR CHALLENGE STUDY

DOG FOOD

H_3PO_4

a_w - 0.85 (28 g H_2O/100g Dog Food)

a_w - 0.88 (45g H 0/100g Dog Food)

CONTROL   INHIBITORS   CONTROL   INHIBITORS

NATURAL   A.NIGER   NATURAL   A.NIGER

5 GRAM SAMPLES STORED at 0.85 a_w

5 GRAM SAMPLES STORED at 0.88 a_w
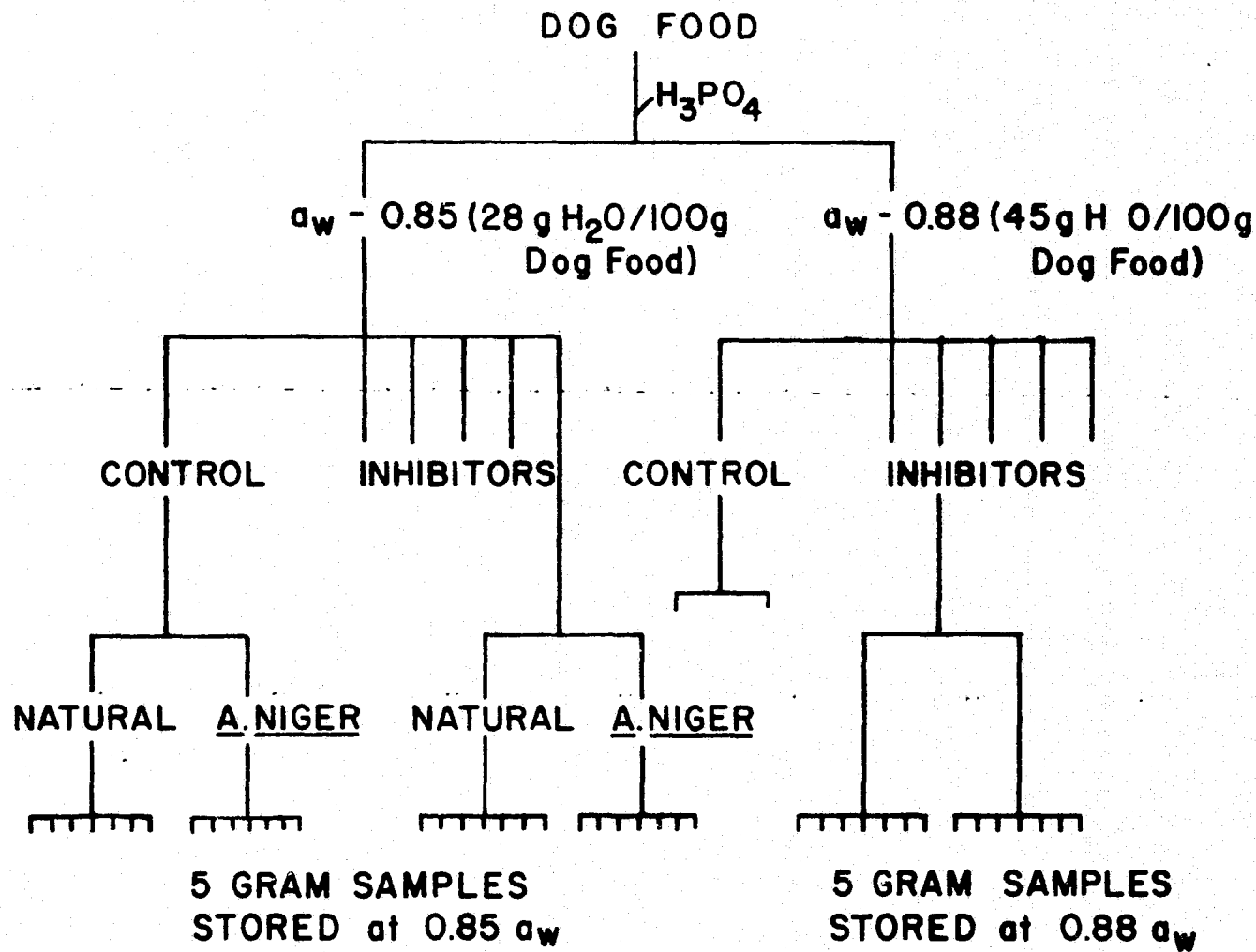
levels tested are presented in Table 1. The chemicals which are not cleared for use in foods were tested in appropriate amounts.

The common food additives, K-sorbate and Ca-propionate, are called acid-type inhibitors as their effectiveness increases as pH decreases (Sauer, 1972). They are usually used in relatively acid foods. The parabens are esters of parahydroxybenzoic acid. This molecular configuration does not have the pH dependence for efficacy as do the acid-type inhibitors (Chichester and Tanner, 1968). Caproic acid has been recommended as a replacement for K-sorbate in IM dog food (Haas, 1973). The antibiotic, pimaricin (Natamycin) is used in European countries in the production of sausage and cheese (Clark et al., 1964). It has selective inhibition of fungal but not bacterial growth.

The polyols are usually used in foods as humectants, plasticizers or emulsifiers. These chemicals also have some degree of antimicrobial action related to the location of the hydroxyl groups and chain length (Patsch and Hoehne, 1969). Some polyols, e.g. glycerol, are metabolized by certain microbes. When this situation exists that polyol will not function as an inhibitory agent. Currently propylene glycol is the most commonly used humectant—antimicrobial agent in semi-moist foods.

The sugars studied are usually employed as sweeteners in foods. They were tested here in their capacity as microbial inhibitors and humectants.

Preparation of inoculum

A. niger was grown for 5 days at 23°C on a 30 ml TSY (Trypticase Soy Agar + Yeast Extract, 0.5%, BBL Brand) agar slant in

TABLE 1

CHEMICAL INHIBITORS IN STUDY

List of Inhibitors Tested	Levels Tested g/100g dog food	Specification
Metabolic Inhibitors:		
K-sorbate	0.3	Anheuser-Busch St. Louis, MO
Ca-propionate	0.3	" "
Parabens, Methyl (USP)	0.3, 0.10	Tenneco Chemical
, Propyl (USP)	0.01, 0.10	Heyden Div, NY, NY
, Combined (2M:1P)	0.05, 0.10	
Caproic acid*	0.1, 0.2, 0.3	
Pimaricin (Delvocid Instant)* (Natamycin)	0.002	Gist-Brocades, N.V. Delft, Holland
Polyols:		
Glycerol	1,2,3,8,10,13	Mallinckrodt #5092 St. Louis, MO
Propylene glycol	1,2,4,6,8,9	" "
Polyethylene glycol 400	16	Union Carbide Chem. New York, NY
1,3 Butylene glycol*	1,2,4,6,10	Celanese Corp, New York, NY
Sugars:		
Fructose	20	J.T. Baker Chem. Glen Ellyn, IL
Mannitol (USP)	1.0	Atlas, ICI America Wilmington, DE
Sorbitol (99% crystalline)	1,4,7,18	" " "

* Not FDA-approved

a cotton-plugged 150 ml prescription bottle. The spores were washed

from the agar with 3—10 ml aliquots of sterile water and gentle shaking

by hand. The population of the suspension was determined by plate

counts on TSY agar. The stock suspension was diluted so that 0.1 ml

in 25 g of test system would give a population of 10^6 CFU/100 g.

Constant vortexing of the suspension was necessary while inoculating

the systems to ensure consistency.

Analytical methods

The pH of the dog food was determined using the gran plot

technique described by Acott and Labuza (1974). To 7 g samples of dog

food, 0.5, 1.0, 30, 5.0 and 7.0 g of water was mixed and equilibrated

at 4°C for 18 hr after warming to room temperature. The pH of the

slurries was determined using an Orion pH meter equipped with a

semi-micro combination electrode (Orion #910200). The pH was plotted

on gran plot paper (Orion Cat. No. 900093, 100% volume corrected)

against the grams of water added to the dog food. Extrapolation to the

pH at zero g of water was taken as the pH of the semi-moist sample.

The pH of the dog food with no acidulant added by the gran plot method

was pH 6.3. This is different than from a direct reading taken on

the semi-moist material (pH 5.9) or when a 1/100 dilution (AOAC, 1970)

was made (pH 7.0). The gran plot method gives a theoretically more

accurate estimation of pH.

The Fett-Vos method (Vos and Labuza, 1974) was used to

determine the a_w of the systems at the initiation of storage. After

6 to 8 weeks of storage the a_w determinations were performed by the

vapor pressure manometric (VPM) procedure (Labuza, 1974) on microbiologically

stable 5 g samples.

The moisture content of the dog food was determined by vacuum drying oven at 29" Hg and $60^{\circ}C$ for 24 hr. The analysis was done on dog food with and without the addition of water. Systems with and without inhibitors added were not used. The loss of polyols during drying would have resulted in erroneous values.

Microbial analysis

The microbial activity was monitored by blending 5 g samples with 45 g of sterile phosphate buffer (0.125%), and spreading 0.1 ml of appropriate dilutions on prepoured TSY agar plates. The plates were inverted and incubated at $23^{\circ}C$ for 5 days in humidified chambers ($a_w = 1.0$). Differential enumeration was based on the morphological differences of the colony forming units. A. niger produced typical black conidia over white spreading mycelia. A. glaucus formed compact masses of mycelia which failed to produce conidia but which were readily distinguishable from bacterial colonies due to the different reflectance of light. The S. epidermitis appeared as typical staph-like, buff-colored, smooth, round colonies. The growth of A. niger and A. glaucus on dog food produced typical conidia, black and green, respectively.

The effective antimicrobial agent was than which prevented the growth of both mold and bacteria. Inhibition of mold growth was determined by sample platings. The antimicrobial agent was deemed unsuccessful when it allowed a two log cycle increase in S. epidermitis population and visible mold growth within 6 months of storage. Conversely, if the organism showed no positive growth the inhibitor was successful.

4. Results and Discussion

The a_w determinations that were done initially and after 6 to 8 weeks of storage indicated a change in a_w during storage in spite of the controlled environment (Table 2). Part of the apparent difference could be due to the use of different methods of a_w determination, as necessitated by the small sample size. Labuza et al. (1975) demonstrated that greater than 0.02 a_w units variability between analytical techniques was common. Specifically, the Fett-Vos Method usually gave values 0.02 units higher than the VPM Method when standardized against saturated salt slurries. This factor alone does not explain the decrease in a_w with storage time. The samples with more of the humectant were closer to the expected a_w's. The sorbitol samples suggest that time was a factor with the a_w decreasing with time. These samples were microbiologically stable, ruling out microbial activity as the cause. The reason for the change may be chemical deterioration. This phenomenon has been observed in other studies (unpublished data). The decrease in a_w should act to make the food more stable to microbial deterioration, however, overall product quality may be a problem. Further consideration of this problem is beyond the scopy of this discussion

The compatability of the three species of microbes is demonstrated by the plate counts of the dog food without inhibitors (Table 3). Initially the occurrence of A. glaucus spores was low, 12 CFU/100 g, and the S. epidermitis population was 10^5 CFU/100 g of dog food. At the end of two weeks at a_w 0.85, A. glaucus and S. epidermitis had reached high populations, 10^8 and 10^7 CFU/100 g, respectively. The growth of A. niger in the challenge system didn't

TABLE 2

A_W HISTORY FOR STORED IMF DOG FOOD

Inhibitor	g/100g Dog food	Initial[1] a_w	a_w of Storage Chamber	a_w after[2] Storage
				VPM
				2 mo.
1,3 Butylene glycol	4	0.88	0.88	0.82
	6	0.88	0.88	0.86
Propylene glycol	4	0.88	0.88	0.83
	8	0.87	0.88	0.87
Glycerol	10	0.85	0.88	0.85
				3 wks.
Sorbitol	4	0.83	0.85	0.82
	7	0.82	0.85	0.80
				6 wks.
Sorbitol	4	0.83	0.85	0.79
	7	0.82	0.85	0.78

(1) Fett-Vos Method

(2) VPM Method

TABLE 3

PLATE COUNTS OF DOG FOOD

NO INHIBITORS ADDED

	Unchallenged		Challenge		
Time	A. glaucus	S. epidermitis	A. niger	A. glaucus	S. epidermitis
0	12	9×10^4	5×10^5	12	9×10^4
4 days	12	8×10^4	4.3×10^5	12	8×10^4
2 wks	9.9×10^7	2.0×10^7	2.0×10^6	5.5×10^7	3.3×10^7

Differential enumeration (CFU/100g dog food)

pH 5.4 (H_3PO_4)

a_w - 0.85

inhibit the growth of the other microbes. Although the $\underline{A}$. $\underline{niger}$ population does not appear to have increased greatly, growth was significant as indicated by the appearance of $\underline{A}$. $\underline{niger}$ on the samples. The compatability of the three species is necessary so that results can be based on the action of the inhibitors and not on that of the challenge organism.

The primary requirement for a microbiologically stable product is the control of the microbe that will initiate growth. Table 4 shows the effect of increased a_w on stability in control dog food and the results when K-sorbate and Ca-propionate were added. At the lower a_w all species had reached high populations in control dog food by two weeks. At the higher a_w, 0.88, the growth rate of $\underline{S}$. $\underline{epidermitis}$ and $\underline{A}$. $\underline{niger}$ was increased. When 0.3% K-sorbate was added to 0.85 a_w dog food, all mold was completely inhibited and the product appeared edible even after 9 months storage. The growth of $\underline{S}$. $\underline{epidermitis}$ was slow but after 25 weeks the population had increased by 2 log cycles. Although there are no "limits" on non-pathogenic staphylococci, the strict criterion of a 2 log cycle increase was used with $\underline{S}$. $\underline{aureus}$ in mind. High populations of this organism when growing at a high enough a_w can produce enterotoxins and thus food poisoning (Troller and Stinson, 1975). At a_w 0.88, $\underline{A}$. $\underline{niger}$ grew in the K-sorbate dog food but $\underline{A}$. $\underline{glaucus}$ was controlled. Haas (1975) found that a variety of this mold was particularly resistant to K-sorbate. The difference in the findings is most likely due to species variation and difference in the growth medium.

Ca-propionate slowed the growth of the molds but after 25 weeks both species were visible at the lower a_w, 0.85. At 0.88 the

TABLE 4

TIME FOR GROWTH OF MICROBES*

Inoculated Dog Food
with Inhibitors, pH 5.4

	Storage Conditions	
Inhibitor	a_w = 0.85 9 mo. storage	a_w = 0.88 6 mo. storage
No Inhibitor added	A. niger - 2wks A. glaucus - 1 wk S. epider. - 2 wks	A. niger - 1 wk A. glaucus - 1 wk S. epider. - ½ wk
K-sorbate (0.3%)	No mold S. epider. - 25 wks	A. niger - 5 wks S. epider. - 3½ wks
Ca-Propionate (0.3%)	A. niger - 25 wks A. glaucus - 25 wks S. epider. - 3½ wks	A. glaucus - 2 wks S. epider. - 1½ wks

* Mold - first visible sign

Bacteria - 2 log cycle increase

growth rate of $\underline{A}$. $\underline{glaucus}$ and $\underline{S}$. $\underline{epidermitis}$ was significantly increased,

and that mold appeared in only 2 weeks. $\underline{A}$. $\underline{niger}$ was completely

inhibited. This data suggests that Ca-propionate should not be

strictly substituted for K-sorbate.

Few of the antimicrobial agents were successful when

tested separately at FDA-approved levels, even at a_w 0.85. Combinations

of these chemicals were tested. The metabolic inhibitors, K-sorbate

or Ca-propionate which were not effective alone, were tested with

appropriate levels of glycerol, propylene glycol, 1,3 butylene glycol,

mannitol and sorbitol.

A summary of the completely successful antimicrobial

systems found in this study is listed in Table 5. The advantage of

using an acidulant is evident in the results for the 0.85 a_w systems.

Only 4% 1,3 butylene glycol was needed at pH 5.4, while more than twice

as much was necessary at pH 6.3. The relative cost of humectants vs.

acidulants makes the use of acidulants quite favorable. The 4%

sorbitol system with 0.3% K-sorbate added was effective as an anti-

microbial system. There may be some advantage to substituting some

sucrose in a product with sorbitol.

By combining inhibitors the dog food system at a_w 0.88

was made shelf stable. No single inhibitor at the levels tested was

effective at this slightly higher a_w. Propylene glycol and 1,3

butylene glycol (4 or 6%) used with K-sorbate or Ca-propionate (0.3%,

maximum allowed by FDA) extended the microbiological shelf life of the

dog food to 6 months. This suggests the use of 1,3 butylene glycol

as a substitute for propylene glycol, although it is not yet cleared

TABLE 5

% LEVELS OF SUCCESSFUL INHIBITORS

AT A_W 0.85

Inhibitor	pH 5.4	pH 6.3
1,3 Butylene glycol	4%	9%
Propylene glycol	--	8.3%
Sorbitol with 0.3% K-sorbate	4%	--

AT A_W 0.88

Inhibitor	pH 5.4
1,3 Butylene glycol with 0.3% K-sorbate	4%
1,3 Butylene glycol with 0.3% Ca-propionate	4%
Propylene glycol with 0.3% K-sorbate	4%
Propylene glycol with 0.3% Ca-propionate	6%

-- = Not tested

by FDA for such use. The results also indicate that Ca-propionate could be used instead of K-sorbate, contradicting the indications taken from Table 4 (i.e. K-sorbate effective against, but Ca-propionate not effective against A. glaucus). This point involves the complexity of the systems and our lack of understanding of interactions therein. Apparently there is a synergistic effect between the polyols and the metabolic inhibitors.

In most cases the results based on challenged and unchallenged systems were the same. Exceptions existed when the naturally occurring mold, A. glaucus, had different sensitivity than A. niger (e.g. K-sorbate at 0.88). Basing the criterion for effectiveness on inhibition of all the test organisms, ensures safety from spoilage by the natural contaminants or by a post-processing contaminant (represented by the organism used in the challenge). Another considerable advantage results from inoculation with a challenge organism, i.e. homogeneous, known initial populations. The results in Table 5 were found for both challenged and unchallenged test systems.

The antimicrobial systems required for stability in this product are more severe than those effective in a similar chicken based IMF system (Acott and Labuza, 1974). This illustrates the importance of product formulation on the degree of antimicrobial protection needed. Furthermore, a slight a_w increase (or equivalent error in a_w determination) can significantly alter the stability of an IMF product.

5. References

Acott, K.M. and T.P. Labuza. 1974. Inhibition of Aspergillus niger in an intermediate moisture food system. J. Food Sci. 40: 137.

Acott, K.M. and T.P. Labuza. 1975. Microbial growth response to water sorption preparation. J. Food Technol. (in press).

AOAC. 1970. "Official Methods of Analysis". Horowitz, W., editor. 11th edition. pg. 214. Assoc. Off. Anal. Chem., Washington, D.C.

Bone, D.P. 1973. Water activity in intermediate moisture foods. Food Technol. 27: 71.

Chichester, D.F. and F.W. Tanner, Jr. 1968. Antimicrobial food additives. In "Handbook of Food Additives". Furia, T.W., editor. pg. 137. Chemical Rubber Co., Cleveland, Ohio.

Clark, W., R. Shirk and E. Kline. 1964. Pimaricin, a new food fungistat. In "4th Int'l Symposium on Food Microbiology", pg. 167. SIK, Goteborg, Sweden.

Haas, G.J. 1973. Canadian Patent #922961. Animal Food Products. Granted to General Food Corp., White Plains, New York.

Haas, G.J., D. Bennett, E.B. Herman and D. Collette. 1975. Microbial stability of intermediate moisture foods. Food Prod. Dev. April.

Labuza, T.P. 1974. Sorption phenomena in foods: Theoretical and practical aspects. In "Theory, Determination and Control of Physical Properties of Food Materials". D. Reidel Co., Dordrecht, Holland. Chapter 10, pg. 119.

Labuza, T.P., S.R. Tatini, K. Acott, R. Lee, J. Flink and W. McCall. 1975. Determination of water activity — A comparative study. Presented at the 35th Annual IFT Meeting, June 9.

Sauer, F. 1972. Control of mold by chemical preservatives. In "Fungi and Food". 7th Annual Symposium, Western New York Section, IFT. October 19.

Troller, J.A. and J.V. Stinson. 1975. Influence of water activity on growth and enterotoxin formation by Staphylococcus aureus in foods. J. Food Sci. 40: 802.

Vos, P.T. and T.P. Labuza. 1974. Technique for measurement of water activity in the high a_w range. J. Agric. Food Chem. 22: 326.

E. Summary and Recommendations

This section has covered the study of the effectiveness of microbial inhibitors in intermediate moisture food systems. It has been found that:

1. Most mold inhibitors are not effective by themselves against <u>Staphylococcus aureus</u>, especially at a_w 0.86 to 0.90 and pH 5.6.

2. Acidification to pH 5.2 improves the effectiveness of mold and <u>S. aureus</u> inhibitors. At this pH most common mold inhibitors also inhibit <u>S. aureus</u>.

3. Pimaricin can be used to prevent mold growth in systems where staphylococcal growth is being studied.

4. In a complex food system containing both molds and staphylococcal species, very few inhibitor systems show complete inhibition at high a_w.

5. Overall a combination of propylene glycol at 4-6% with either K-sorbate or calcium propionate (0.1 to 0.3%) would seem to be totally effective as a microbial inhibitor system at pH 5 to 6.

III. Effect of Water Activity on the Death Rates of Pathogenic

Organisms as a Function of Water Activity

A. Introduction

It is well-known that water activity plays an important role in the heat resistance of microbial spores and vegetative cells. Water activity alone, however, cannot fully explain the variation in decimal reduction time of the same microorganism in different media held at the same temperature, such as was shown by Baird-Parker et al. (1970). Actual foods contain various solutes, salts and acids which can exert different effects on the growth, survival and/or toxin production of microorganisms as suggested by Labuza (1973). The heat resistance of microorganisms may also be influenced. Since changes in the heat resistance of either spoilage or pathogenic microorganisms will greatly affect the calculation of heat processing time for foods, it is essential to find out the decimal reduction time of microorganisms in the foods, not in a synthetic media. In this section both liquid media and a semolina-egg dough mixture were studied as a function of a_w.

Study of the heat resistance of microorganisms in intermediate moisture foods is a formidable task. Two prerequisites for successful microbial destruction tests are (1) a uniform distribution of microorganisms and (2) uniformity of temperature throughout the food under investigation. In liquid media, these can be achieved easily by using a stirrer and a constant temperature bath. It is apparent that this technique is not appropriate for intermediate moisture foods since they are highly viscous and heat transmission is slow. Some of

the designs which can be applied in solid food systems, i.e. capillary tube methods, can method, metal cup method and cup-thermoresistometer methods were briefly sumamrized by Pflug et al. (1968). What should be stressed is that while each method has certain advantages and disadvantages, none can completely satisfy all prerequisites. The individual parts of this section discuss these problems.

With respect to a_w, maximum heat resistance for vegetative cells has been repeatedly found in the IMF water activity range (0.75—0.80) (Elizondo, 1973; Corry, 1974; Labuza, 1974). The reason for this, however, has never been fully explained. For example, are the mechanisms of death or inactivation of cells the same at both high and low a_w? Elizondo (1973) proposed that the decrease in death rate at high a_w was due to the decrease of aqueous phase reaction rates when water becomes limiting. At low a_w, however, the death was probably due to the combination of osmotic and temperature stress. Gibson (1973) found that marked volume reductions in cells occurred when a_w was lowered from 1.0 to 0.85 as seen through photographic and O.D. measurements. She suggested that an almost instantaneous dehydration of the protoplasm occurred, followed by shrinkage of the whole cell when cells were placed into hypertonic solution. Thus, the decrease in death rate when a_w is lowered from 1.0 to 0.85 resulted from:

1. The decrease of the cell water content which in turn confers some protection against heat to the cell protein.

2. The reduction in the pore size of the cell wall, thus minimizing the loss of intracellular components on heating.

Through freeze-etch electron microscopy, Corry (1974) noticed that glycerol caused very little or no plasmolysis effect in salmonellae which was very different from that produced by sucrose, glucose, sorbitol or fructose at the same a_w. Although the water content of the protoplasm would be reduced in all the solutes used, she found that the more severe the plasmolysis was, the greater was the protection to the cells during heating. Thus, the value of the decimal reduction time D of salmonellae decreased in the order: sucrose > glucose > sorbitol > fructose > glycerol.

Another direction which no one has ever exploited is to find the relationship between activation energy of microbial death or inactivation of cells and a_w. Although equivalent activation energy is not necessarily a positive implication of the same mechanism of reaction, different activation energies do provide an indication of possible different mechanisms of death. Some assumptions should be made here.

1. The thermal inactivation of microorganisms follows a first order reaction, i.e.

$$-\frac{dN}{dt} = kN$$

where N = number of cells present at time t
k = death rate constant

2. The death rate constant k can be related to the absolute temperature T through the Arrhenius equation:

$$k = k_0 \exp (-Ea/RT)$$

where k = death rate constant
k_0 = frequency factor
Ea = activation energy of microbial death
R = universal gas constant
T = absolute temperature

Table I shows the activation energies in solid foods and
liquid media. In general, the activation energy of microbial death
for solid foods is somewhat higher than that for liquid media.
Charm (1958) indicates that although Ea was essentially constant
with temperature, different media could result in different activation
energies. Furthermore, the activation energy at low a_w is less than
that at high a_w on the order of about 10-20 Kcal/g mole. This is
quite significant since it implies that the mechanism of death at
high a_w is not likely to be the same as that at low a_w. This study
will investigate this hypothesis.

References

Baird-Parker, A.C., M. Boothroyd and E. Jones. 1970. The effect of
    water activity on the heat resistance of heat sensitive and heat
    resistant strains of salmonellae. J. Appl Bacteriol. 33: 515.

Charm, S.E. 1958. The kinetics of bacterial inactivation by heat.
    Food Technol. 12: 4.

Corry, J.E.L. 1974. The effect of sugars and polyols on the heat
    resistance of salmonellae. J. Appl. Bacteriol. 37: 31.

Elizondo-Ruiz, H. 1973. Death kinetics of microorganisms during
    dehydration. M.S. Thesis, Univ. of Minnesota, St. Paul.

Gibson, B. 1973. The effect of high sugar concentration on the heat
    resistance of vegetative microorganisms. J. Appl. Bacteriol.
    36: 365.

Kemeny, G. and B. Rosenberg. 1973. Compensation law in thermo-
    dynamics and thermal death. Nature 243: 400.

Labuza, T.P. 1973. Storage stability and improvement of intermediate
    moisture foods. Contract NAS 9-12560. Phase I. NASA. Houston.

Labuza, T.P. 1974. Storage stability and improvement of intermediate
    moisture foods. Contract NAS 9-12560. Phase II. NASA. Houston.

Peri, C. and L. DeCesari. 1974. Thermodynamics of water sorption
    on Saccharomyces cerevisiae and cell viability during spray-
    drying. Lebensmittel-Wissenschaft + Technol. 7: 76.

TABLE 1

INACTIVATION ENERGIES FOR MICROORGANISMS

Organisms	Media	Temp. range °F	Inactivation energy	Ref.
S. aureus 196E	0.5% NaCl	140-155	89.50	(5)
	Skim milk	" "	101.11	"
	Beef bouillon	" "	88.19	"
	Green pea soup	" "	114.07	"
S. aureus MS149	0.5% NaCl	140-155	70.49	(5)
	Skim milk	" "	85.39	"
	Beef bouillon	" "	91.88	"
	Green pea soup	" "	112.68	"
S. seftenberg 775W-R	0.5% NaCl	140-160	80.72	(5)
	Skim milk	145-165	84.42	"
	Beef bouillon	140-160	87.21	"
	Green pea soup	140-160	92.82	"
S. seftenberg 775W-S	0.5% NaCl	140-155	86.33	(5)
	Skim milk	145-155	89.06	"
	Green pea soup	140-155	86.25	"
Bacillus stearothermophilus (FS 7954) spores	Phosphate buffer	127.2-143.8	83.60	(6)
P.A. 3679 spores	Pureed peas	220-270	32.40	(1)
" " "	Strained squash	" "	30.50	"
	Cooked fresh pork	" "	30.50	"
	White sauce	" "	30.50	"
	Distilled water	" "	31.80	"
Clostridium botulinum spores	Pureed peas	220-260	38.00	(1)
	Strained squash	" "	35.80	"
	Distilled water	" "	36.40	"
Staphylococcal enterotoxin B	Raw milk	210-260	25.90	(3a)
	0.04M Veronal buffer	96-126.7	20.70	(3b)
Staphylococcal enterotoxin A (or D)	Milk	100-121.1	25.40	(4)
Saccharomyces cerevisiae ATCC 9763-1N	10% Lactose (w/v)	70-80	14.20	(2)
		80-105	1.05	"

(1) Charm, 1958
(2) Peri et al, 1974
(3) Read et al, 1966a, 1966b
(4) Soo, 1974
(5) Thomas et al, 1966
(6) Wang et al, 1964

Pflug, I.J. and C.F. Schmidt. 1968. "Thermal destruction of
     microorganisms". In Disinfection, Sterilization and Preservation.
     C.A. Lawrence and S.S. Block, editors. Lea and Febiger Pub.
     Philadelphia. pp. 80-83.

Read, R.B., Jr. and J.G. Bradshaw. 1966a. Thermal inactivation of
     staphylococcal enterotoxin B in Veronal buffer. Appl.
     Microbiol. 14: 130.

Read, R.B., Jr. and J.G. Bradshaw. 1966b. Staphylococcal entero-
     toxin B thermal inactivation in milk. J. Dairy Sci. 49: 202.

Rosenberg, B., G. Kemeny, R.C. Switzer and T.C. Hamilton. 1971.
     Quantitative evidence for protein denaturation as the cause
     of thermal death. Nature 232: 471.

Soo, H.M. 1974. Thermal inactivation of staphylococcal enterotoxin
     A and D in certain food products. M.S. Thesis, Univ. of
     Minnesota, St. Paul.

Thomas, C.T., J.C. White and K. Lungree. 1966. Thermal resistance
     of salmonellae and staphylococci in foods. Appl. Microbiol.
     14: 815.

Wang, D.I.C., J. Scharer and A.E. Humphrey. 1964. Kinetics of
     death of bacterial spores at elevated temperatures. Appl.
     Microbiol. 12: 451.

B. The Effect of Water Activity on the Heat Resistance of

Vegetative Cells in the Intermediate Moisture Range

Reprinted on the following pages is the article published

in Lebensmittel-Wissenschaft + Technology 8: 78-81 (1975).

The Effect of Water Activity on the Heat Resistance of Vegetative Cells in the Intermediate Moisture Range

F. Hsieh, K. Acott, H. Elizondo and T. P. Labuza

Department of Food Science and Nutrition,
University of Minnesota—St. Paul
St. Paul, Minnesota 55108 (U.S.A)

The death rate of microorganisms as a function of water activity and temperature is a parameter necessary for development of safe food processes. In certain processing, such as drying or extrusion, both water activity (a_w) and temperature change simultaneously. The results of this study show that at pasteurization temperatures in liquid media, the death rate for vegetative cells including pathogens is at a minimum in the intermediate moisture range of a_w (0.75–0.85). Thus, moist food components should be pre-pasteurized before combining with a_w lowering agents.

Introduction

The lowering of moisture content and water activity has a pronounced effect on the heat resistance of microbes. There is much evidence in the literature to support this. TROLLER (11) showed this in a review on bacterial pathogens. MURRELL and SCOTT (10) found that the heat resistance of *Clostridium botulinum* spores in the dry state was greater than when held in water vapor at an a_w of 1. However, the maximum resistance to heat occurred in the a_w range of 0.2 to 0.4. No theoretical basis could be found for this maximum, however, it must be related to the monolayer value. HÄRNULV and SNYGG (5) found that above a concentration of 55.8% glucose solution, spores of *Bacillus subtilis* had an increase in heat resistance. This would correspond to a_w 0.85.

Salmonella species, because of their pathogenicity, have been the subject of much research with respect to a_w. GOEPFERT et al. (4) found that for seven *Salmonella* serotypes and *Escherichia coli* the heat resistance increased with a decrease in a_w to 0.75. On the other hand, BAIRD-PARKER et al. (2) showed a decreased resistance for certain strains at about a_w 0.94. At lower a_w, however, the heat resistance increased. Decreased heat resistance occurred only in the very high a_w range. KADAN et al. (6) have also shown this reversal in the heat resistance of *Staphylococcus aureus* in sugar solutions. Heat resistance decreased as sucrose increased to 14%; above that (up to 57% sucrose gave a protective effect. GIBSON (3) also showed a decrease in heat resistance in vegetative cells down to about a_w 0.84 with a slight reversal. The reasons for the change in heat resistance have never been explained and make it difficult to predict death of microbes as a function of a_w, especially during spray drying and extrusion processing

of foods in which a_w and temperature can be continuously changing. The purpose of this study was to determine the effect of a_w on the death of vegetative cells over a wide range of water activities.

Studies were made on the effect of glycerol as an a_w lowering agent on the heat resistance of *Saccharomyces cerevisiae*. *Salmonella anatum* and *Staphylococcus aureus*. This was done to determine, over a wider range of a_w, the effect of a_w on heat resistance of vegetative cells. These results could be used to verify the predictions made by LABUZA et al. (8) for death during processing of foods containing pathogenic vegetative cells. In addition, if pathogens have a heat resistance similar to yeast, the effect of simultaneous drying and heating on death could be studied without the need of a sealed pathogen room.

Materials and Methods

Test Procedure

Saccharomyces cerevisiae ATCC 7754 was grown in nutrient broth (Difco) at 21 °C on a shaker at 300 rpm for 48 hr. The cells were concentrated by centrifugation, washed and suspended to give a count of about 10^9 colony forming units/ml. The test medium (99.9 ml) comprised of a mixture of glycerol and sterile skim milk at the desired a_w was transferred into a sterile 125 ml flask in a model FK2 Haake water bath. A sterile magnetic stirring bar was put into the flask to facilitate mixing. Four temperatures were used (25, 55, 57 and 60 °C ± 0.05). After temperature equilibration. 0.1 ml of the suspension was introduced directly into the test solution by means of a 100 μl liquid syringe to give an initial count of 10^6 to 10^5 CFU/ml. At selected times 0.1 ml was removed, diluted in phosphate buffer and plated by the surface spread technique on potato dextrose agar (Difco) and counted after 48 hr at 35 °C. The death rate constant k (min⁻¹) was calculated as the slope of the straight line found. This is theoretically equal to 2.3 times the reciprocal of the decimal reduction time.

The same technique was used for the two pathogens, *S. aureus* 196E and *S. anatum* NF₃. These were grown for 24 hr at 37 °C in 100 ml trypticase soy broth and 0.5% yeast extract (TSYB), centrifuged, suspended to give a population of around 10^9 CFU/ml. The thermal inactivation study was carried out in glycerol-brain heart infusion (BHI, BBL brand) broth with an initial population of 10^6 CFU/ml. Samples were taken at 5 min intervals, diluted in 0.1% peptone water (Difco), the organisms were plated by the surface spread technique on

Tab. 1 Depression of a_w in Test Systems by Glycerol

Glycerol Percent by Weight	Glycerol-BHI Measured a_w	Glycerol-Milk Mixture Measured a_w
0	0.99	0.99
20	0.95	–
30	–	0.87
40	0.87	–
50	0.83	0.76
60	0.77	–
63	–	0.67
70	0.68	–
75	–	0.50
80	0.53	–
82	–	0.47
90	0.35	0.29
95	0.24	–

Tab. 2 Composition of Glycerol-Peptone Water Dilution Blanks at Different a_w Levels

a_w	ml of glycerol	ml of peptone water
1.00	0	9.0
0.95	1.5	7.5
0.87	2.4	6.6
0.83	4.0	5.0
0.77	4.91	4.09
0.68	5.86	3.14
0.53	6.86	2.14
0.35	7.90	1.10
0.24	8.44	0.56
0.01	9.0	0

TSYA, and enumerated after 18 hr at 37 °C. The data were treated the same way to get *D*, the decimal reduction time, and *k*, the death rate constant.

Test Solutions

Sterile skim milk (9% solids Mid America Dairy) or BHI was mixed with glycerol in the combinations shown in Tab. 1. The a_w was measured directly in the vapor pressure manometer. A water activity below about 0.29 could not be used in the heat resistance studies of *S. cerevisiae* because the high viscosity of the solution prevented rapid mixing with the magnetic stirrer. In the studies of *S. aureus* and *S. anatum* this was overcome by using a Corning LM-2 electric stirrer.

Investigation of Dilution Stress on Cell Death

Preliminary studies on death of vegetative cells have indicated that the dilution blank used might have some effect on the viable counts of dehydrated cells from spray drying. Supposedly, to detect the effect of a stress treatment, no further stress should be imposed on the cells after the treatment in the medium. Thus, the dilution blank should be cooled for heat-treated cells. Following this, it seems in order that some osmotic stress or shock might occur in plating cells that are heated at various a_w's if the a_w of the dilution blank is different from that of the heating medium. With this in mind, an experiment was conducted to determine the significance of the "dilution blank effect".

A set of glycerol-peptone water dilution blanks were made. It was assumed that the same weight percentage of glycerol in both BHI and peptone water would give the same water activity. Tab. 2 shows the composition of the 9 ml glycerol-peptone water dilution blanks at various water activities. *S. anatum* NF₃ was chosen for this study as it is the least resistant to a_w stress. The organism was introduced at 10^6 CFU/ml into the BHI-glycerol solutions at 52 °C and an initial sample and samples for every five min heating time were taken. Appropriate dilutions were made in both peptone water and glycerol-peptone water using a dilution blank at the same a_w as the heating medium and plated immediately by the surface-spread technique on TSYA plates. The plates were inverted and enumerated after incubating 18 hr at 37 °C.

Results and Discussion

Tab. 3 shows that the initial count decreases steadily with the increase in weight percentage of glycerol in the glycerol-BHI heating solution based on a decrease in water activity. As seen, when glycerol is not used in the dilution blank, at low a_w's

Tab. 3 Effect of Dilution Blank on Death of *Salmonella anatum* Introduced into Different Solutions

	Relative Initial Count*		
a_w of Glycerol-BHI solution a_w	Peptone water dilution blank	Glycerol-peptone water dil. blank	Ratio of counts in glycerol-pep. to pep. H₂O dil. blank
1.00	1	1	1
0.95	0.833	1	1.20
0.87	1.083	1.083	1
0.83	0.708	0.691	0.976
0.77	0.60	0.817	1.361
0.68	0.342	0.517	1.512
0.53	0.275	0.508	1.848
0.35	0.175	0.342	1.952
0.24	0.0667	0.242	3.625
0.01	0.0342	0.208	6.098

* = ratio to count at 0% glycerol

there is an immediate two- to six-fold increase in death. This indicates the sum of all the stresses including osmotic shock and suggests the possible need for use of proper dilution blanks. The data also indicate that stress occurs without heating, since the data are from zero time at 52 °C.

Tab. 4 shows the ratio of the D-values (decimal reduction times) at 52 °C as a result of using the two different dilution blanks. As seen, at high a_w the difference is small with a higher death rate in the non-glycerol dilution system as expected. The difference becomes more pronounced at lower water activity, but is always less than one logarithm cycle. Thus, even though a dilution stress exists, its effect is not as large as first thought when comparing death rates. Thus, death rate studies were made without the use of the a_w controlling dilution blanks since most research has not used that technique.

The results of the thermal inactivation studies on *S. cerevisiae*, *S. anatum* and *S. aureus* are shown in **Fig. 1**, **2** and **3**. An increase in k means greater death rate (smaller decimal reduction time). From the pure skim milk or BHI medium, as glycerol is added (decrease in a_w to 0.76-0.85) the heat resistance increases for all the test organisms. This is as would be expected for spores and was also found for the pathogenic organism studies cited in the introduction. However, at lower a_w the heat resistance decreases.

ACKER (1) has shown that the rate of enzymatic reactions decreases as a_w is decreased. LABUZA (7) has shown this for other reactions. Possibly the binding of water reduces the rate of the reaction causing death, or the glycerol itself stabilizes any labile macromolecules since it helps to structure water. Glycerol itself does not cause death of the cells in this water

Tab. 4 D-Values for Different Dilution Blanks at 52 °C for *Salmonella anatum* F_a*

a_w at 33 °C	D_1 (min) Peptone water dilution blank	D_2 (min) Glycerol-sterile H_2O dilution blank	D_2/D_1
0	6.8	8.8	1.79
0.55	11.8	22.0	1.86
0.73	14.6	39.0	2.67
0.77	25.7	36.3	1.41
0.87	20.7	28.0	1.35
0.95	10.4	10.4	1.0
1.00	3.10	3.10	1.0

* (based on one run)

Fig. 1 Rate constant (min⁻¹) for death of S. cerevisiae as a function of a_w in glycerol-milk solutions as determined from heating curves at three temperatures

Fig. 2 Rate constant (min⁻¹) for death of S. anatum as a function of a_w in glycerol-BHI solutions as determined from heating curves at three temperatures

Fig. 3 Rate constant (min⁻¹) for death of S. aureus as a function of a_w in glycerol-BHI solutions as determined from heating curves at three temperatures
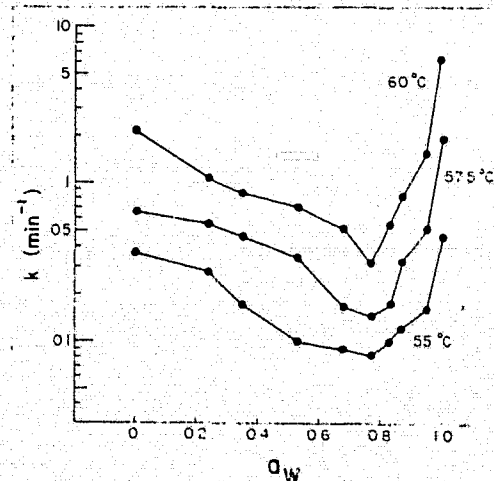
Tab. 5 Effect of Glycerol on *Saccharomyces cerevisiae* at 25 °C (Viable Cells per ml)

Incubation time	Control skim milk	a_w of Glycerol-Milk Test Solutions					
		0.87	0.76	0.67	0.50	0.47	0.29
Zero	5.5×10^4	6.0×10^4	5.2×10^4	3.5×10^4	3.9×10^4	3.1×10^4	2.7×10^4
1 min	- -	6.0×10^4	5.0×10^4	3.4×10^4	3.9×10^4	3.0×10^4	2.7×10^4
3 hour	5.5×10^3	6.2×10^3	4.6×10^3	1.5×10^4	5.1×10^3	6.4×10^2	4.0×10^1
24 hour	6.6×10^5	3.8×10^3	6.8×10^3	2.3×10^2	< 30	0	0

activity range (0.99–0.75) as seen in **Tab. 3** at 52 °C and in **Tab. 5** at 25 °C. The diffusion of glycerol into the cells should not be much greater at 50–60 °C than at 25 °C since diffusion rate increases as a function of the square root of temperature in absolute degrees.

Below a_w 0.75, the heat resistance decreases significantly becoming close to that at the high a_w (0.99). In this region, the glycerol itself may be having an effect on the cell. This is obvious for the salmonellae at high temperature (52 °C), as seen in **Tab. 3**, since the counts immediately began dropping rapidly. **Tab. 5** shows these results for the yeast at 25 °C, however, the effect does not become obvious until an a_w below 0.5 is reached after 3 hr holding time, a time much longer than that used in the heating studies. Thus, some other factor such as osmotic stress in combination with the heat stress decreases the cell resistance.
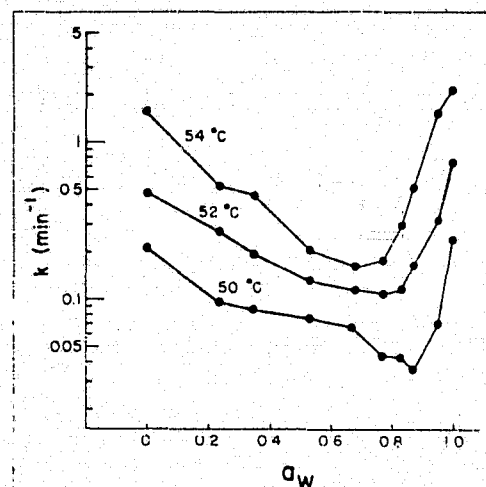
As noted, MURRELL and SCOTT (10) found a maximum in heat resistance for spores at 0.2–0.4 a_w. Other work with vegetative cells never covered the full range, so only the increase in resistance was shown down to an a_w of about 0.74–0.84 where the maximum was found in this study. Possibly the difference in the maxima between the studies could be due to a sorption hysteresis effect. The spore study was done with dried spores re-equilibrated to different a_w's on the adsorption branch of the isotherm whereas the yeast study would constitute a desorption isotherm. LABUZA et al. (9) have shown that sorption hysteresis can have an effect on the limiting a_w for microorganisms. This could partially account for the difference found. Further work is needed to clarify this. Another important factor as seen in the figures is that the yeast used dies at a rate about ten times faster than the pathogens. This suggests, therefore, that yeast cannot be used in extrusion or drying studies as an indicator as had been hoped.

Conclusion

This study shows that for vegetative cells including the pathogenic species *S. anatum* and *S. aureus*, the heat resistance is at a maximum value in the intermediate moisture range for liquid medium. Although this has not been studied in a solid food system, it would be expected that the same conditions would exist. Thus, it is recommended that components used to make intermediate moisture foods such as meat, eggs, etc. which are at high a_w should be heat pasteurized prior to combination with the a_w lowering agents and other dry food components. This would insure maximum kill and minimum use of energy during final processing.

References

1 ACKER, L.: Recent Advances in Food Science. Vol III. pp. 239–247. Butterworth's, London (1963)
2 BAIRD-PARKER, A. C., BOOTHROYD, M. and JONES, E., J. Appl. Bacteriol. 33, 515 (1970)
3 GIBSON, B., J. Appl. Bacteriol. 36, 365 (1973)
4 GOEPFERT, J. M., ISKANDER, I. K. and AMUNDSON, C. H., Appl. Microbiol. 19, 429 (1970)
5 HARNULV, B. G. and SNYGG, B. G., J. Appl. Bacteriol. 35, 615 (1972)
6 KADAN, R. S., MARTIN, W. H. and MICKELSEN, R., Appl. Microbiol., 11, 45 (1963)
7 LABUZA, T. P., Proc. III Int'l Congress Food Science & Technol., SOS/70, pp. 618–635 (1971)
8 LABUZA, T. P., LEROUX, J. P., FAN, T. S. and TANNENBAUM, S. R., Biotech. & Bioeng. 12, 135 (1970)
9 LABUZA, T. P., CASSIL, S. and SINSKEY, A. J., J. Food Sci., 37, 160 (1972)
10 MURRELL, W. G. and SCOTT, W. J., J. Gen. Microbiol., 43, 411 (1966)
11 TROLLER, J. A., J. Milk Technol., 36, 276 (1973)

C.   Death Kinetics as a Function of a_w in a Solid Food System -

Preliminary Tests

1.   Introduction

The Brabender Farinograph has been used extensively
in the cereal industry to study dough characteristics as a function
of temperature and composition.   It is very useful because of the
rapid mixing and supposedly uniform component and temperature
distribution.   This preliminary study was designed to test whether
this instrument could be used for a death kinetics study in solid
food systems.

2.   Methods

a.   Temperature distribution

The solid food system used was semolina with 5.5%
whole egg solids.   The water content of the mix was adjusted such that
the water activity ranged from 0.51 to 0.93.   Six thermocouple probes
(two J-SS4-G-PJF-4", Conax Corp., Buffalo, New York and four SCPSS-
062G-6, Omega Engineering, Inc., Stanford, Conn) were used.   Five of
them were evenly distributed from the wall to the center of the bowl
in which the mix was contained, and one was used to measure the ambient
temperature.   The dough was added and the water in the jacket was
turned on.   The temperature profile was read from the Leeds and Northrup
Speedomax Multipoint Recorder.

b.   Microorganism distribution

A culture of S. aureus 196E was prepared by inoculating
0.1 ml of fresh subculture into 100 ml of TSYB with glass beads and
incubating at room temperature ($23^{o}C$) on a shaker for 24 hr.   Final

population of the stock culture varied between 10^9 - 10^{10} organisms

per ml. Thus, each stock culture was plated at the beginning of each

run. Initially, 99 g of the flour mix was prepared according to the

isotherm and was placed in the Brabender bowl. The motor was

started and 1 ml of the culture (10^9 - 10^{10} organisms per ml) was

added. At 2, 4, 6 and 8 min, approximately 1 g of sample was taken

out and weighed into the blender jar which contained 99 ml of peptone

water. The exact weight of the sample was recorded. Appropriate

dilutions were made and plated in TSYA by the surface spread technique.

Plates were inverted and incubated at 37°C and enumerated after 24

hr.

3. Results and Discussion

As seen in Figure 1, the temperature profile of the food

systems in the Brabender Farinograph bowl was remarkably flat, i.e.

the temperature of the system in the bowl was constant regardless of

the position where the thermocouple probe was installed. Initially,

one to two degrees Farenheit difference was observed. After about four

min, the bulk of the system showed essentially the same temperature.

The difference between the temperature of the wall and the food as seen

in Table 1 varied from 0.5°F to 5°F increasing steadily as the a_w

increased. This was due to the air film which existed between the wall

and the food system and was also influenced by the size of food

granules. It seems that eleven to twelve min was always necessary for

the food temperature to become stable and a good even distribution is

obtained.

As seen in Table 2 for the microbial distribution study,

the number of microorganisms per gram of sample fluctuates somewhat

FIGURE 1

TEMPERATURE DISTRIBUTION IN BRABENDER BOWL WITH SEMOLINA EGG DOUGH

T_w = wall
T_c = center
T_a = ambient
T_1 = between center and wall
T_2 and T_3 (not drawn) fall
    between T_1 and T_c
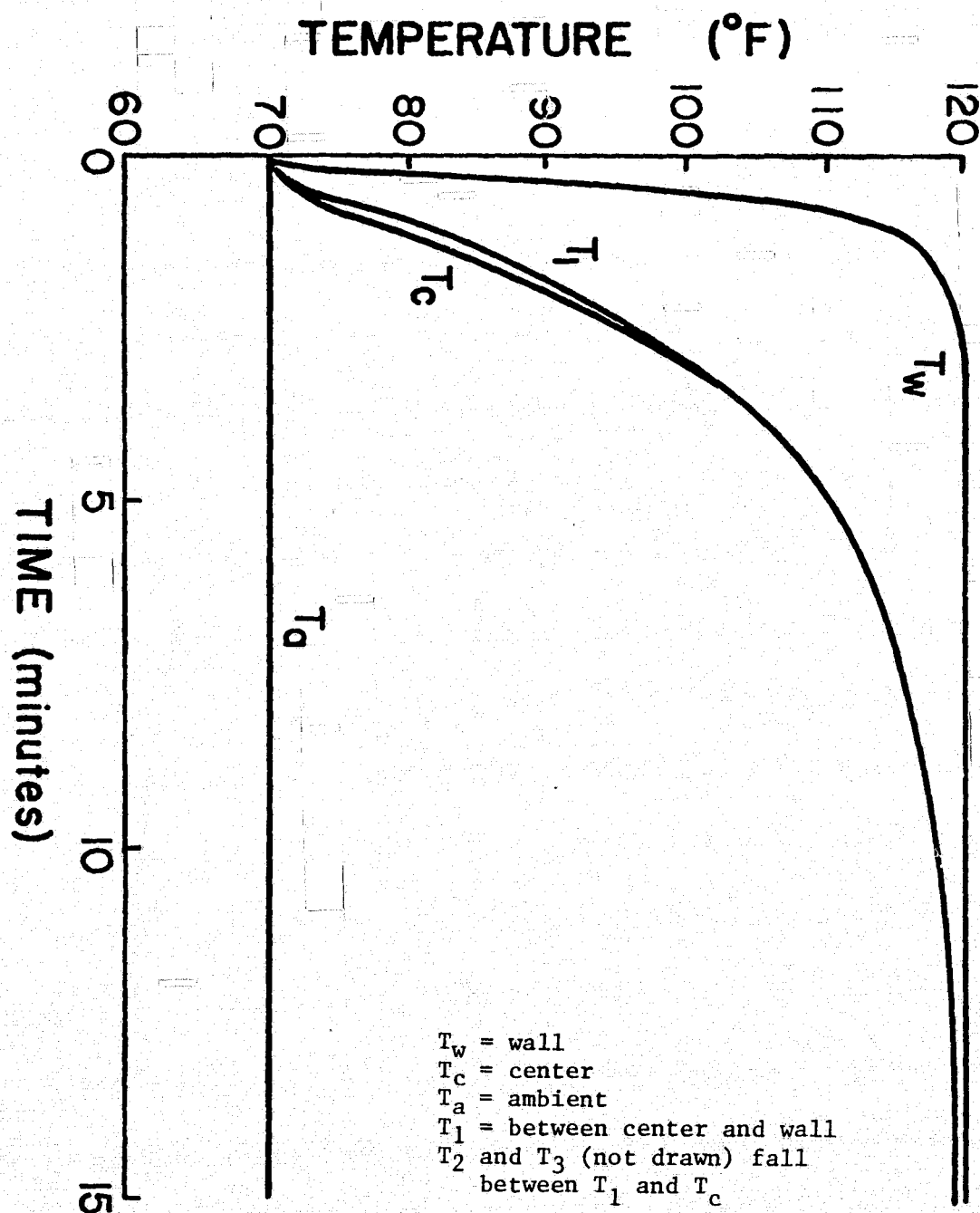
TABLE 1

TEMPERATURE STUDY OF A SOLID FOOD SYSTEM
DURING MIXING IN THE BRABENDER FARINOGRAPH BOWL

Water activity	0.51	0.75	0.82	0.86	0.90	0.93
Time for solid food system to reach stable temperature (minutes)	12	12	12	12	12	11
Final wall temperature (^{O}F)	119	119	119	119.5	120	120
Final food system temp. (^{O}F)	118.5	118.5	118.5	118.5	119	115
Jacket water temp. (^{O}F)	122	122	122	122	122	122
Ambient air temp. (^{O}F)	72	70	71	70	70	74

TABLE 2

TIME FOR MIXING AND DISTRIBUTION OF MICROORGANISMS

a_w	Time of mixing	Weight of sample (g)	Number of organisms	Number of org./ml	Number of org./g
	Stock			1.3×10^9	
0.63	2 min	1.1	7.2×10^6		6.5×10^6
	4 min	0.9	1.3×10^7		1.4×10^7
	6 min	1.1	2.2×10^7		2.0×10^7
	8 min	1.5	5.5×10^7		3.7×10^7
	Stock			4.6×10^9	
0.76	2 min	0.9	3.4×10^7		3.8×10^7
	4 min	1.1	4.8×10^7		4.4×10^7
	6 min	1.0	4.7×10^7		4.7×10^7
	8 min	1.7	9.3×10^7		5.5×10^7
	Stock			7.1×10^9	
0.87	2 min	0.9	7.5×10^7		8.3×10^7
	4 min	1.1	5.0×10^7		4.5×10^7
	6 min	1.2	1.3×10^8		1.1×10^8
	8 min	0.9	9.6×10^7		1.1×10^8
0.93	2 min	1.6	1.3×10^8		8.1×10^7
	4 min	1.35	1.1×10^8		8.1×10^7
	6 min	1.2	1.0×10^8		8.3×10^7
	8 min	1.35	1.1×10^8		8.1×10^7

around the expected dilution of the stock. Two min of mixing time

apparently is almost enough time to give uniform distribution of

microorganisms in the Brabender bowl, especially at high a_w conditions.

While the count fluctuates quite a bit at a_w 0.63, it is surprisingly

constant at the higher a_w tested. It seems that a lower a_w requires

a longer time for mixing than the higher a_w's.

Although small granules or pockets did form during the

drop-wise addition of 1 ml inoculum, the subsequent mixing in the

Brabender bowl gave reasonably uniform distribution. Also, the fairly

large amount of sample (approximately 1 g) contributed to the constancy

of the results that were found in this study. The thermal destruction

study of microorganisms will be conducted at $50^{o}C - 60^{o}C$, not at

extremely high temperatures. A one or two log cycle of death may

occur before uniform distribution is achieved, however, it will still

be possible to find the decimal reduction time with an initial population

of approximately 10^7 organisms/ml.

D.   Death Kinetics of Food Pathogens in a Solid Food System

Reprinted on the following pages is a copy of the article

submitted to the Journal of Food Science for publication. The

paper was presented at the 35th Annual IFT Meeting.

DEATH KINETICS OF FOOD PATHOGENS

IN A SOLID FOOD SYSTEM

Fu-hung Hsieh, K. Acott, T.P. Labuza

Department of Food Science and Nutrition
University of Minnesota—St. Paul
St. Paul, Minnesota 55108

1. Abstract

The death kinetics of _Salmonellae anatum_ NF3 and _Staphylococcus aureus_ 196E was determined as a function of temperature and water activity. (a_w). Death kinetics was measured in a solid medium composed of a semolina-egg dough which was mixed at constant temperature in a Brabender Farinograph bowl. First order death kinetics was found at all conditions. The results showed that a maximum in resistance to heat occurred in the range of a_w of 0.75 to 0.80 for both organisms with a 4 to 10 fold increase in resistance respectively. These results show that to insure safety from pathogens, intermediate moisture food components may have to be pasteurized before mixing to the lower a_w.

2. Introduction

Numerous studies have been carried out to investigate the water relations of microorganisms (Scott, 1957; Troller, 1973). Most of them were basically concerned with the water activity (a_w) below which growth would not occur or the rate of growth as a function of a_w. Another area of importance is the effect of a_w on the death rate of microorganisms as a function of temperature. While the former areas are significant in the storage stability of foods, the latter area is an important parameter for the development of safe food processes. This is because the time-temperature relationship in all pasteurization and sterilization processes is governed by the heat resistance of microorganisms.

With respect to bacterial spores, Murrell and Scott (1966) found that the greatest heat resistance was manifested at a_w values of about 0.2 to 0.4 when a_w was controlled from the vapor state. Angelotti et al. (1968) also reported that maximum heat resistance was observed within the same range of a_w. Alderton and Snell (1970) found that the optimal a_w for maximal survival of the spores was 0.28 after heat treatment. Pace et al. (1972) showed continually decreasing survival of spores with increasing a_w from 0.5 to 0.9. All these results are consistent with those of Murrell and Scott (1966). This was further confirmed for bacterial spores by Harnulv and Snygg (1972) in studies where a_w was controlled by both vapor and aqueous solution systems.

With respect to vegetative cells, Goepfert et al. (1970) found that the heat resistance of salmonellae always increased as the a_w of the heating menstruum was reduced. Unfortunately, the range of a_w studied was only from 0.87 to 0.99 for sucrose solution due to the limitation of solubility and

and 0.75 to 0.99 for aqueous glycerol. Gibson (1973) showed that maximum

heat resistance occurred about 0.70 to 0.80 a_w for vegetative cells of

bacteria and yeasts in aqueous sucrose or sucrose and glucose solutions.

Elizondo (1973) and Hsieh et al. (1975) reported that the heat resistance

was enhanced in this same range of a_w for baker's yeast in glycerol-skimmilk

solutions and for Staphylococcus and Salmonella species in glycerol-Brain

Heart Infusion solutions. Corry (1974) also found that maximum heat resistance

of salmonellae in aqueous glycerol was at 0.6 to 0.8 a_w.

In general, therefore, spores exhibit maximum heat resistance around

a_w of 0.2 to 0.4, while vegetative cells are most heat resistant in the inter-

mediate moisture food water activity range (0.65-0.90). The reasons for the

changes in heat resistance with a_w and the apparent different responses

between spores and vegetative cells have never been fully understood. It also

should be pointed out that most of the cited studies only report either the

percent of survival after a certain period of heating time or D-values at

one temperature level. Moreover, these studies were generally conducted in

a synthetic liquid medium. Thus, the results have had very little use for

realistic food processes such as extrusion and drying in which both a_w and

temperature can change simultaneously.

Of major interest among various food processes in the manufacture of macaron

products. Walsh (1972) has reviewed the bacteriological aspects, specifically the

samonella and staphylococcus problems, of pasta processing. Lee et al. (1975)

reported that routine surveillance by the Food and Drug Administration showed

Staphylococcus aureus contamination of some pasta products manufactured in

the United States. The widespread consumption of these products and the in-

creased number of seizures and recalls which involved microbiologically

contaminated macaroni products (Walsh et al., 1974) suggest more extensive investigation is needed to determine the effect of pasta processing on death rate of microorganisms. The purpose of this study was to determine the effect of a_w on the heat resistance of two representative food pathogens over the ranges of a_w and temperature for the drying of macaroni products. The temperatures of concern are $120-160°$ ($50-65°C$) which is the range of most macaroni drying operations and a_w's of 0.93 to 0.6 which is the range that occurs in drying.

3. Materials and Methods

The organisms used were Staphylococcus aureus 196E and Salmonella anatum NF_3. They were grown in TSYB (Trypticase soy broth (BBL) with 0.5% yeast extract (BBL)) at room temperature on a shaker for 24 hours. The final population was about $10^9 - 10^{10}$ organisms per ml.

The medium for the thermal destruction test was a typical extruded food — an egg macaroni product. For easier mixing and preparation, the raw materials — semonlina (Como No. 1 Semolina, Capitol Durum Division, International Multi-foods Corp., Mpls. MN) and whole egg solids (A.J. Pietrus & Sons Co., Sleepy Eye, MN) — were used instead of the finished product. The ratio of semolina to egg solids was determined first to meet the Standard of Identity (Anon., 1973) of 5.5% whole egg solids. Different amounts of water were then added to reach various water activities, which were measured by the vapor pressure manometric technique (Labuza, 1973). The isotherm of the solid medium is shown in Figure 1.

For thermal destruction tests in the solid medium, a water jacketed Brabender

FIGURE 1
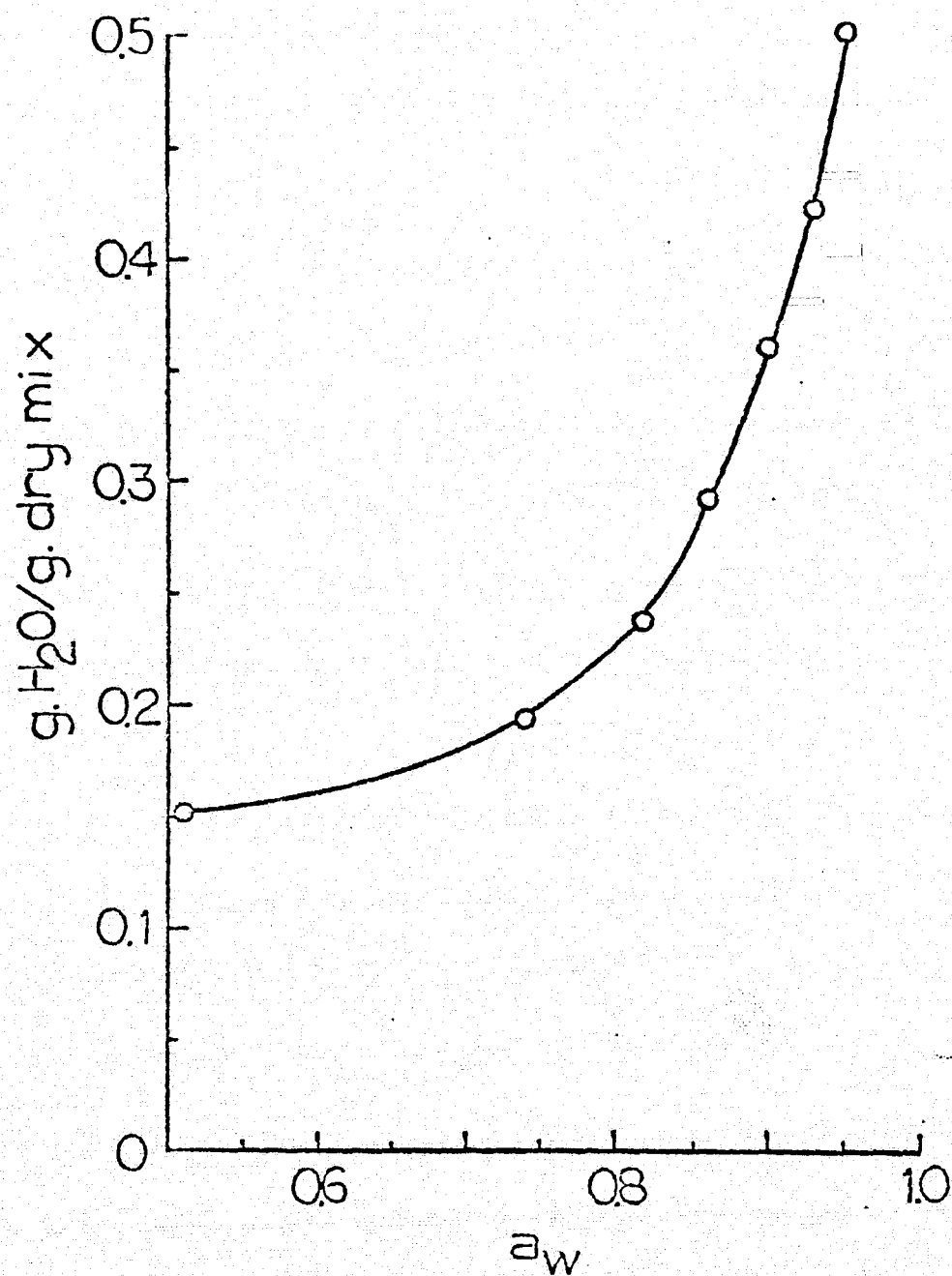
MOISTURE SORPTION ISOTHERMS OF SEMOLINA-EGG DOUGH

MIX AT 35°C

Farinograph bowl (C.W. Brabender Instruments, Inc., South Hackensack, N.J.) was used. This was connected to a constant temperature circulating water bath (Haake FK2, Haake Instruments, Inc., Saddle Brook, N.J.) and outfitted with stainless steel sheathed, sub-minature thermocouple probes (SCPSS - 062G-6, Omega Engineering, Inc., Stanford, Conn.) to measure the medium temperature in the bowl. It was found that 5 to 6 minutes were necessary to achieve uniform distribution of microorganisms after addition of 1 ml inoculum while 12 to 13 minutes were needed to bring the medium temperature up to constant temperature. No temperature gradient was found within the medium after steady state was reached. The thermal destruction tests were conducted after uniform distribution of organisms in the medium was ensured and the medium temperature was at steady state. At preselected time intervals, approximately 1 g of sample was taken out and weighed into a blender jar which contained 99 ml. of 0.1% peptone water. The exact weight of the sample was recorded. The sample was then blended well and appropriate dilutions in peptone water were made and plated immediately by the surface spread technique on TSYA (Trypticase soy agar (BBL) with 0.5% yeast extract (BBL)). These plates were incubated at $37^\circ C$ for at least 24 hours before enumeration.

4. Results and Discussion

All the survivor curves were plotted as log N/N_o (where N_o is the number of vegetative cells at zero time and N is the number of survivors at various heating times). Some typical results are shown in Figure 2 for S. aureus 196E and Figure 3 for S. anatum NF_3. The decimal reduction time D was obtained from the linear portion of the survivor curve. The reciprocal of the decimal
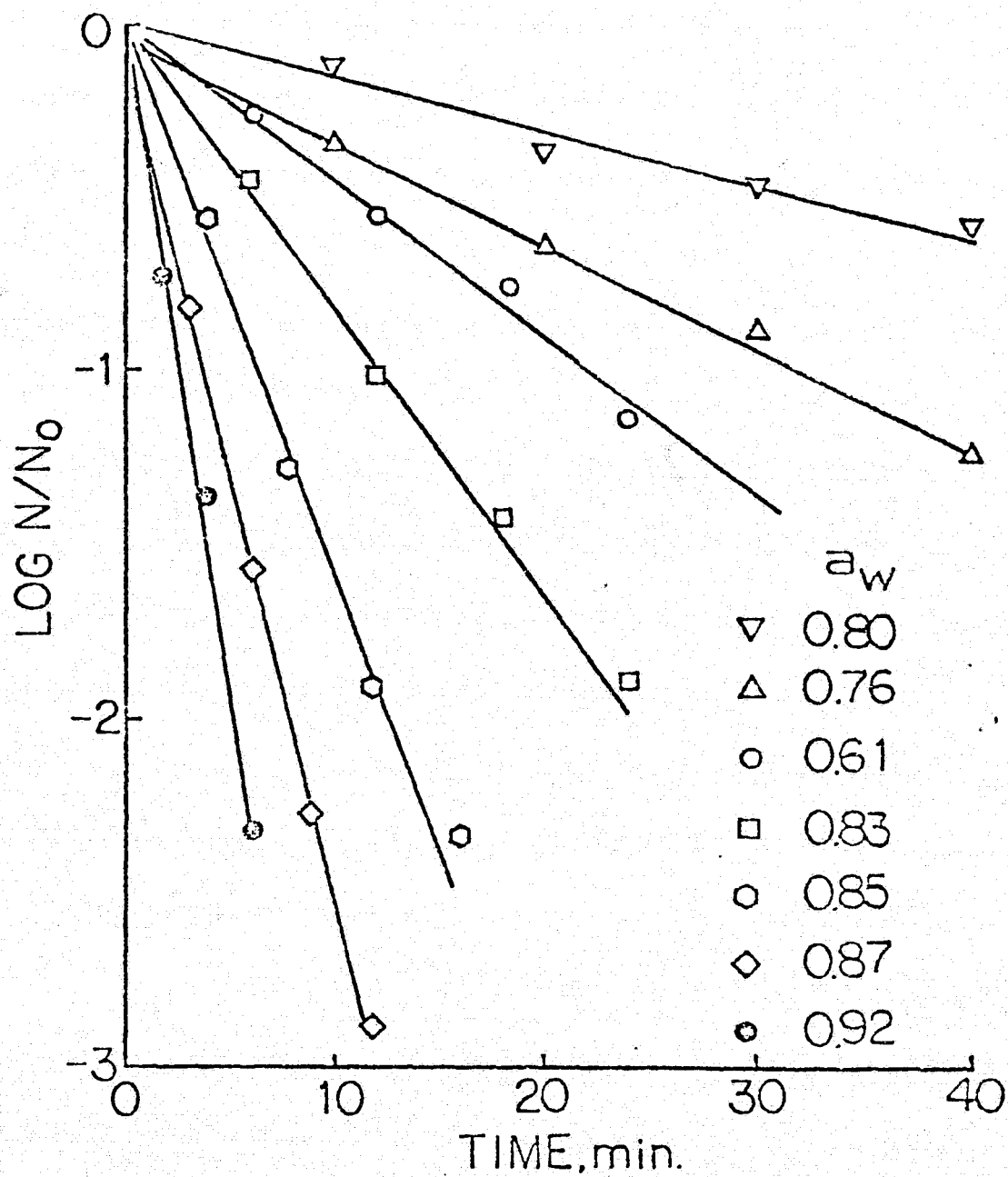
FIGURE 2

SURVIVOR CURVE FOR S. aureus 196E IN SEMOLINA-EGG DOUGH
AT 60°C AS A FUNCTION OF WATER ACTIVITY
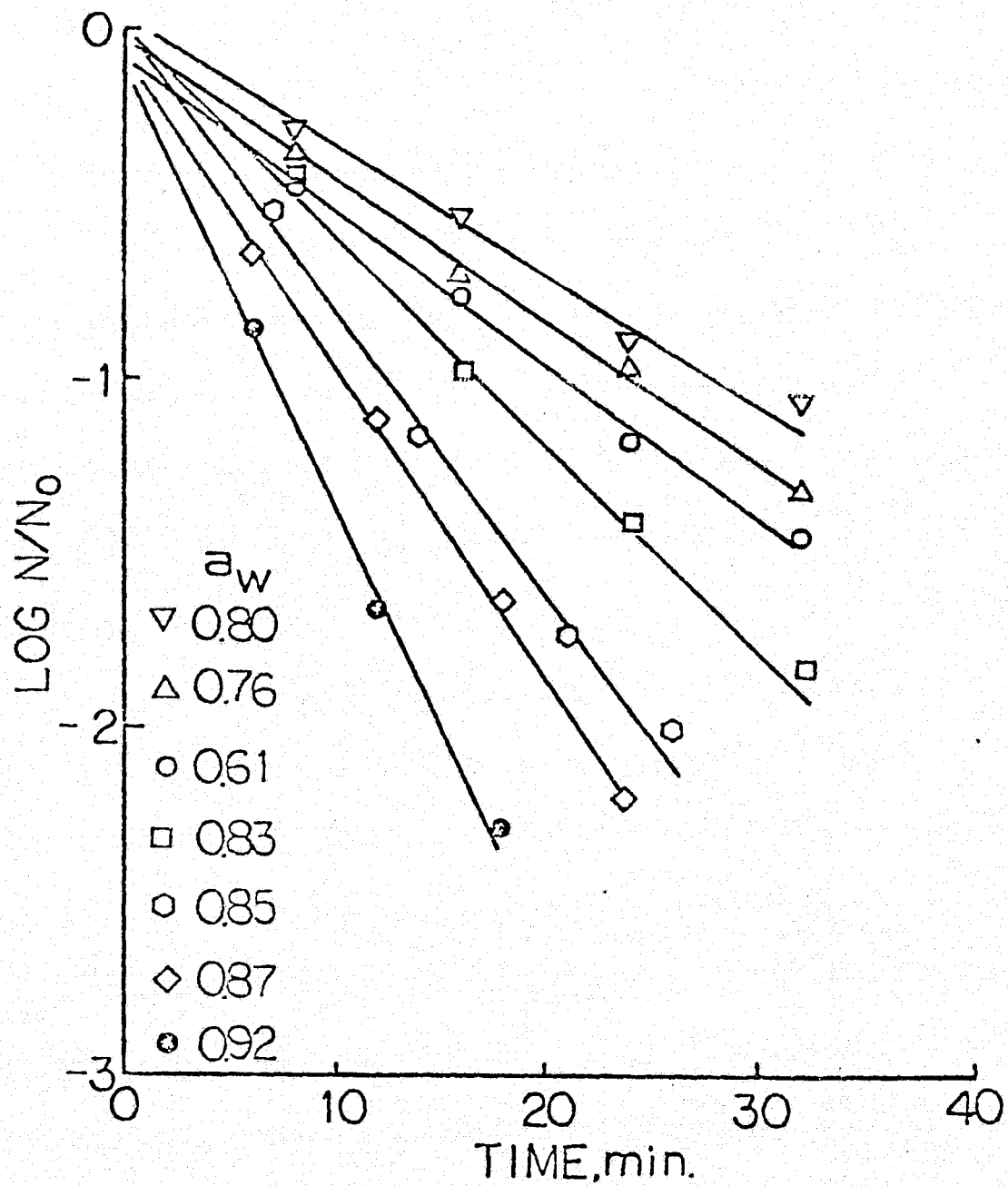
FIGURE 3

SURVIVOR CURVE FOR S. anatum NF₃ IN SEMOLINA-EGG DOUGH

AT 54°C AS A FUNCTION OF WATER ACTIVITY

reduction time times 2.3 gives the death rate constant k. The relationship between the heat resistance and water activity is shown in Figures 4 and 5 for S. aureus 196E and S. anatum NF$_3$, respectively.

Remarkable changes in the heat resistance of both S. aureus 196E and S. anatum NF$_3$ are observed when the a_w of the solid medium is lowered from 0.92 to that of the intermediate moisture food range. Since k is inversely proportional to D, the decimal reduction time, a decrease in k means a lower death rate. As seen in the range of 0.92 to 0.8 about a 4-fold decrease in the death rate occurs for S. anatum NF$_3$ and a 10-fold decrease in the death rate occurs for S. aureus 196E. Drastic changes in heat resistance of vegetative bacterial cells have been noted by Gibson (1973) and Corry (1974). Both of them found more than 10-fold changes in heat resistance for salmonellae in this range of a_w's. It seems that both species and strains of organisms play a role in determing the changes of heat resistance with a_w. This was also noted by Goepfert (1970).

It is also interesting to compare the results of this study with those of a previous study using a synthetic liquid medium, BHI-Glycerol (Hsieh et al., 1975). The k versus a_w values from both liquid and solid media are plotted at one temperature as shown in Figures 6 and 7 for S. aureus 196E and S. anatum NF$_3$, respectively. As seen, both pathogens show more heat resistance in solid media than in liquid media at the same a_w. In addition, both the a_w at which the maximum heat resistance occurs is slightly higher for solid medium than liquid medium. A small decrease in a_w of the solid medium is possible during the thermal destruction study because of water evaporation. However, this cannot fully explain the difference. The major reason is probably due to the nature of the medium. Presence of discontinuities (Mate, 1965) and formation of agglomerates (McDonough and Hargrove, 1968) or other factors which are not
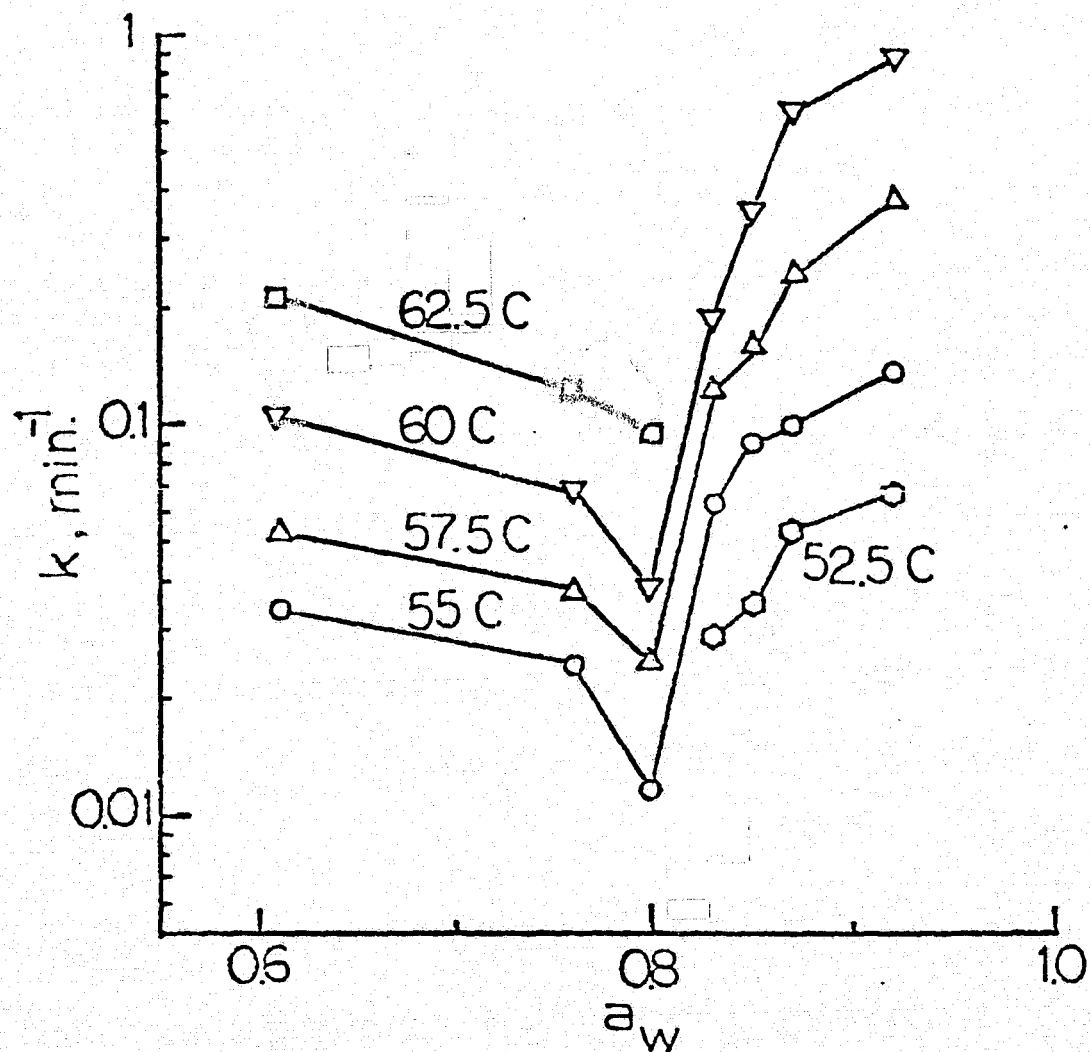
FIGURE 4

DEATH RATE CONSTANT k FOR S. aureus 196E IN SEMOLINA-
EGG DOUGH MIX AS A FUNCTION OF a_w AND TEMPERATURE
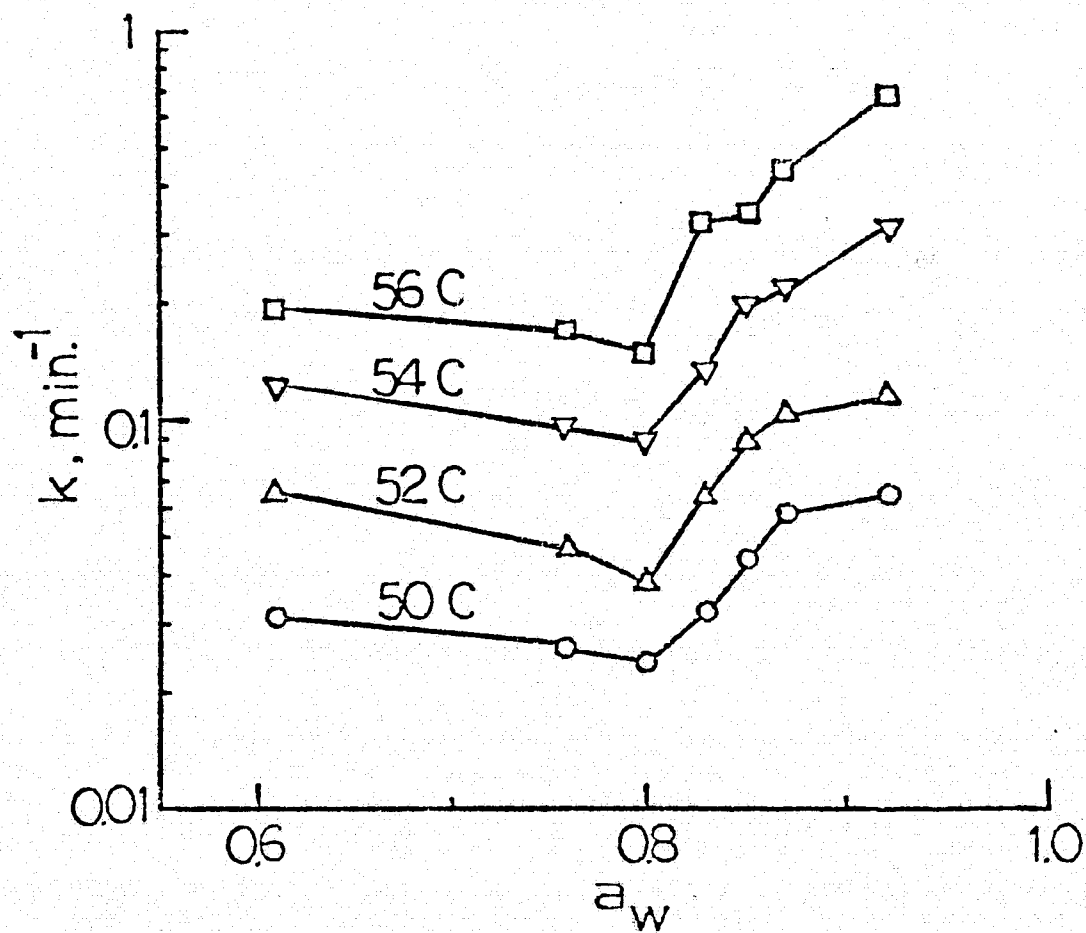
FIGURE 5

DEATH RATE CONSTANT k FOR <u>S. anatum</u> NF_3 IN SEMOLINA-
EGG DOUGH MIX AS A FUNCTION OF a_w AND TEMPERATURE
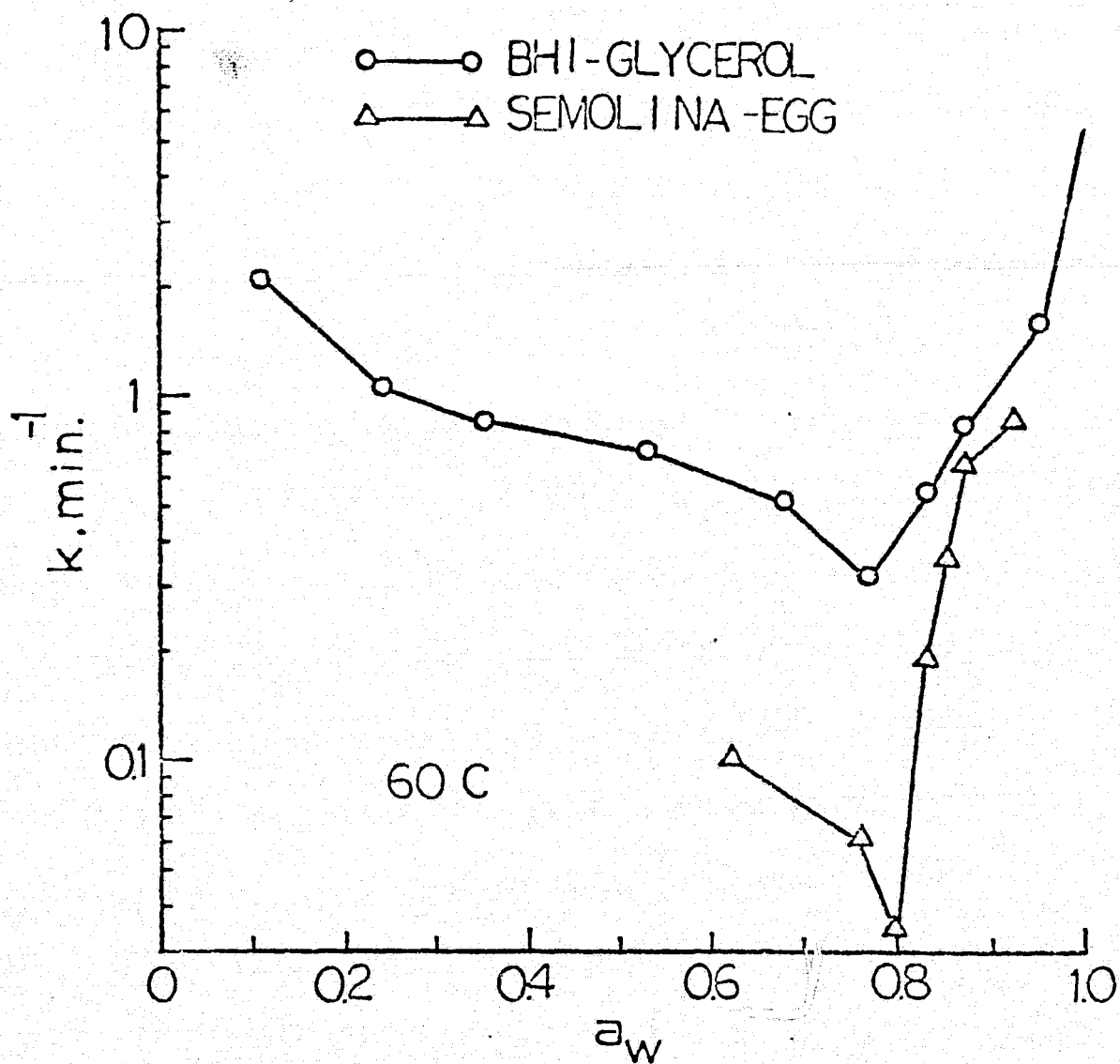
FIGURE 6

COMPARISON OF DEATH RATE CONSTANT k FOR $\underline{S}$. $\underline{aureus}$ 196E IN

BRAIN-HEART INFUSION-GLYCEROL WITH THAT IN SEMOLINA-EGG

DOUGH AS A FUNCTION OF a_w AT 60°C
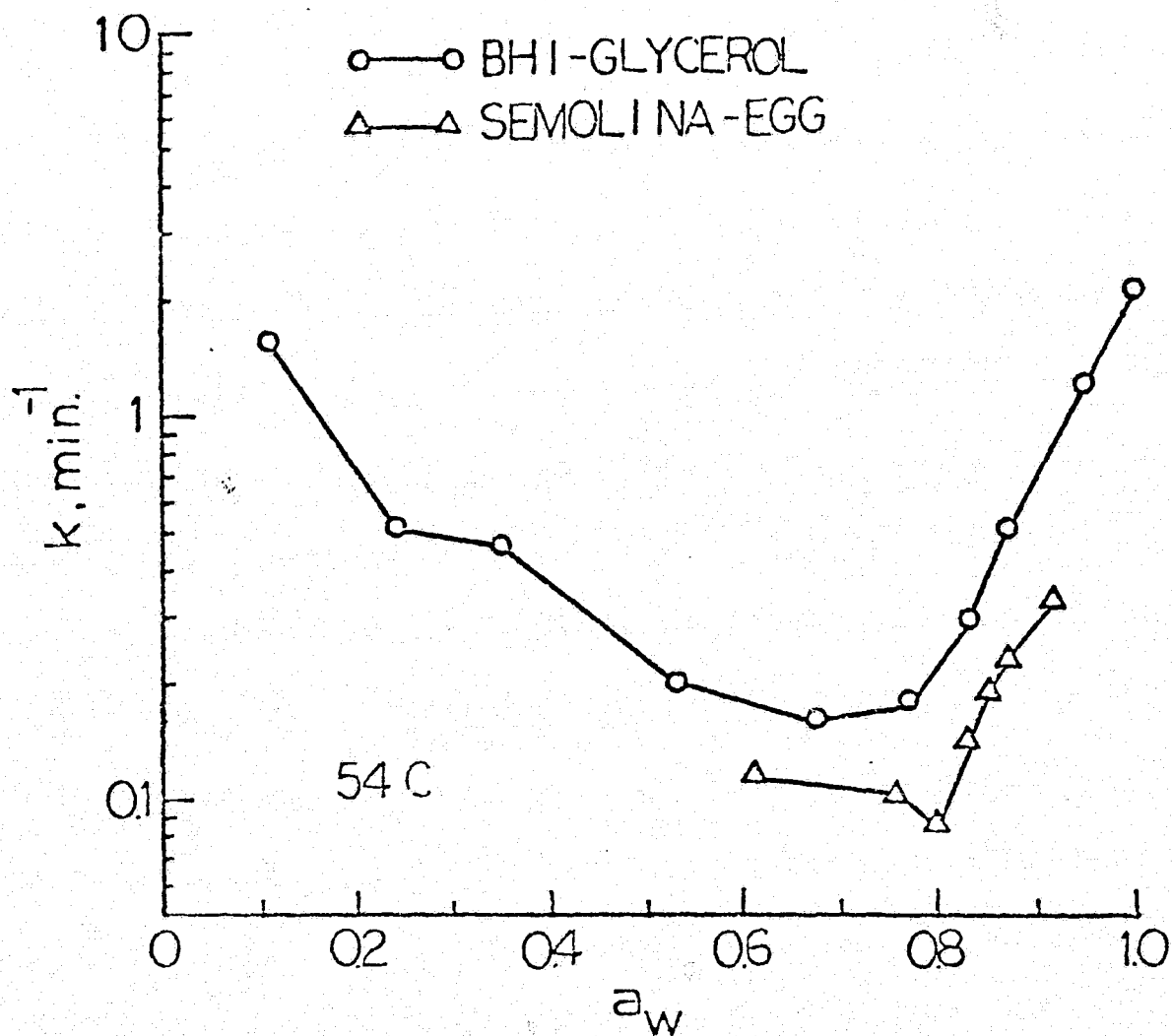
FIGURE 7

COMPARISON OF DEATH RATE CONSTANT k FOR S. anatum NF$_3$ IN

BRAIN-HEART INFUSION-GLYCEROL WITH THAT OF SEMOLINA-EGG

DOUGH AS A FUNCTION OF a$_w$ AT 54°C

directly related to the measured a_w are also suggested.

The phenomenon that the heat resistance of vegetative cells increases when a_w is lowered from 1.0 to that of IMF range have been reported by many others (Riemann, 1968; Goepfert et al., 1970; Baird-Parker et al., 1970; Elizondo, 1973; Gibson, 1973; Corry, 1974; Hsieh et al., 1975). The results made no difference whether a_w was controlled by water vapor or by adding a_w lowering agents, such as glycerol, sucrose, glucose or NaCl to the heating medium. The reason for this has been discussed by Gibson (1973), Elizondo (1973) and Hsieh et al. (1975) and reviewed by Hsieh (1975). Further research is still needed in this area to explain the reasons. However based on these results it can be seen that a maximum in heat resistance occurs in the IMF range.

5. Conclusions

This study shows that the death rate of pathogenic vegetative cells including both salmonellae and staphylococci is at a minimum in the intermediate moisture range of a_w around 0.8 at pasteurization temperatures (50-65°C) for solid medium. Although similar phenomenon was also shown for liquid medium, both the heat resistance and the rate of change in the heat resistance with a_w is different for different medium. Thus, the heat resistance of micro-organisms in real foods cannot be inferred directly from data collected from tests in other liquid or synthetic medium but must be experimentally determined in that particular food.

Because of an increase in the heat resistance when a_w is lowered, it seems advisable to pasteurize the food at high a_w whenever it is feasible.

This will ensure adequate pasteurization with minimum cooking or processing. The data collected in this study are also very useful for prediction of the death of organisms during processing of macaroni products in which changes in a_w and temperature are known.

6. References

Alderton, G. and N. Snell. 1970. Chemical states of bacterial spores: heat resistance and its kinetics at intermediate water activity. Appl. Microbiol. 19: 565.

Angelotti, R., J.H. Maryanski, T.F. Butler, J.T. Peeler, J.E. Campbell. 1968. Influence of spore moisture content on the dry heat resistance of Bacillus subtilis var. niger. Appl Microbiol. 16: 735

Baird-Parker, A.C., M. Boothroyd and E. Jones. 1970. The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of salmonellae. J. Appl. Bacteriol. 33: 515.

Corry, J.E.L. 1974. The effect of sugars and polyols on the heat resistance of salmonellae. J. Appl. Bacteriol. 37: 31.

Elizondo-Ruiz, H. 1973. Death kinetics of microorganisms during dehydration. M.S. Thesis. University of Minnesota.

Gibson, B. 1973. The effect of high sugar concentrations on the heat resistance of vegetative microorganisms. J. Appl. Bact. 36: 365.

Goepfert, J.M., I.K. Iskander and C.H. Amundson. 1970. Relations of the heat resistance of salmonellae to the water activity of the environment. Appl. Microbiol. 19: 429.

Harnulv, B.G. and B.G. Snygg. 1972. Heat resistance of Bacillus subtilis spores at various water activities. J. Appl. Bacteriol. 35: 615.

Hsieh, F., K. Acott, T.P. Labuza, 1975. The effect of water activity on the heat resistance of vegetative cells in the intermediate moisture range. Lebensmittel-Wiss. + Technol. 8: 78.

Hsieh, F. 1975. Death kinetics of food pathogens as a function of water activity. Ph.D. Thesis. University of Minnesota.

Labuza, T.P. 1973. Storage stability and improvement of intermediate moisture foods. Contract NAS 9-12560, NASA. Houston, Texas.

Lee, N.H., C.L. Staples and J.C. Olson, Jr. 1975. Staphylococcus aureus growth and survival in macaroni dough and the persistance of enterotoxins in the dried products. J. Food Sci. 40: 119.

Matz, S.A. 1965. Water in foods. In The Relations of Microbial
     Spoilage to Water Activity of Foods. AVI Publishing Co., Westport,
     Conn.   Chapter 18.

McDonough, F.E. and R.E. Hargrove. 1968. Heat resistance of
     salmonellae in milk. J. Dairy Sci. 51: 1587.

Murrell, W.G. and N.J. Scott. 1966. The heat resistance of bacterial
     spores at various water activities. J. Gen. Microbiol. 43: 411.

Pace, P.J., E.R. Krumbiegel and H.J. Wisnienski. 1972. Interrelation-
     ship of heat and relative humidity in the destruction of
     Clostridium botulinum type E spores on whitefish chubs. Appl.
     Microbiol. 23: 750.

Riemann, H. 1968. Effect of water activity on the heat resistance of
     salmonella in dry materials. Appl. Microbiol. 16: 1621.

Scott, W.J. 1957. Water relations of food spoilage microorganisms.
     Adv. Food Res. 7: 83.

Troller, J.A. 1973. The water relations of food-borne bacterial
     pathogens: A review. J. Milk & Food Technol. 36: 276.

Walsh, D.E. 1972. Bacteriological aspects of pasta processing. (The
     salmonellae and staphylococcal problems). Macaroni J. 54: 16.

Walsh, D.E., B.R. Funke and K.R. Graalum. 1974. Influence of spaghetti
     extruding conditions, drying and storage on the survival of
     Salmonella typhimurium. J. Food Sci. 39: 1105.

E. Summary and Recommendations

   1. Water activity has a definite effect on the heat resistance of vegetative cells for both liquid media and solid foods.

   2. While the death rates of cells are about the same for both systems at high a_w, they are quite different in the IMF range. The largest difference occurs at around a_w 0.80. When a_w is lowered further, the difference diminishes again.

   3. In general, S. aureus 196E is more heat resistant in a solid food than in liquid media.

   4. There consistently exists a maximum heat resistance of vegetative microorganisms in the a_w of IMF range for both liquid media and solid food.

   5. Based on this, food ingredients should be pasteurized before mixing together for IMF processing.

IV. Non-Enzymatic Browning in IMF

A. Introduction

Non-enzymatic browning is one of the most prevalent deteriorative

reactions in intermediate moisture foods. It is basically a reaction

between proteins and reducing compounds resulting in a dark pigment,

loss of protein solubility, bitter flavors and destruction of lysine

which is a primary reactant. Much effort has been centered around

finding an agent or formulation method to control non-enzymatic browning,

especially since it proceeds at a very rapid rate in the IMF water

activity range. In addition, a rapid biological test method is needed

to determine loss of nutritional value.

The results of this section are divided into the following

areas:

1. Effect of protein substitution on the rates of non-enzymatic

browning

2. Effects of humectants on non-enzymatic browning

3. Development of a rapid biological test method for determining

lysine loss

These three areas were considered important since in Phase II

of this contract our work showed that to minimize browning, reducing

sugars should be kept to a minimum. Thus it was felt necessary in

addition to determine the effect of specific proteins with sugars. The

results also showed that glycerol increased browning at low a_w but

decreased it at higher a_w. Based on this, research was carried out to

determine the basis of this effect as well as to determine in addition

the effect of other glycols. Lastly initial work showed large lysine

losses by chemical analysis occurred even before visual pigment appeared. Thus it was felt that a biological confirmation of this was necessary. Unfortunately, the standard rat PER studies are expensive and not suited to this type of research. Thus a rapid microbiological test was evaluated.

B. Effect of Protein Substitution on the Rate of Non-Enzymatic Browning – Analytical Details

1. Introduction

The results of this study are contained in the paper in this section which was submitted to the Journal of Food Science. Since the analytical details are not presented extensively in the paper nor do they appear in the previous final contract reports, they are contained in the following section.

2. Methods

a. Non-enzymatic browning pigment production

Non-enzymatic browning was determined by a method which was modified from that of Choi et al. (1949) as follows:

(1) Suspend 2.0 g of sample in 2.5 ml trypsin solution (10% w/w) and 20 ml phosphate buffer solution (pH 7.8).

(2) Incubate at 45°C for 2 hr at 120 CPM agitation.

(3) Denature trypsin by adding 2.0 ml 50% (w/w) trichloro acetic acid solution.

(4) Add 0.1 g celite filter aid.

(5) Filter contents through What #1 filter paper.

(6) Read absorbance of filtrate against reagent blank (abs. 420 nm).

b. Lysine determination

Available lysine was determined by the Booth modification (1971) of the Carpenter method fluoro-dinitro benzene (FDNB) procedure as follows:

(1) Weigh out 0.8—0.9 g of sample into a 100 ml boiling flask.

(2) Add 4-6 anti-bump boiling chips to each flask.

(3) Add 10 ml NaHCO$_3$ solution to each flask.

(4) Add 15 ml FDNB solution to each flask (FDNB solution is 0.4 ml FDNB/15 ml ETOH).

(5) Stopper flasks and shake for 2 hr at room temperature at 120 CPM.

(6) Boil for 30 min at 82-85°C.

(7) Cool with ice to prevent frothing.

(8) Add 30 ml 8.1 N HCL.

(9) Reflux for 16 hr.

(10) Rinse condensors with a minimum amount of deionized water.

(11) Filter <u>HOT</u> through Whatman #541 filter paper into 250 ml volumetric flasks.

(12) Wash filter paper with deionized water until no residual yellowness remains.

(13) Bring to 250 ml with deionized water.

(14) Shake and let settle approximately 1 hr.

(15) Pipet 2 ml into 1-10 ml volumetric test tube marked A and two tubes marked B.

TUBE A

(16) Wash 3 times with 5 ml diethyl ether. Each
time removing excess ether with a pipet attached to an aspirator.

(17) Boil off remaining ether in boiling water bath.

(18) Cool and bring to 10 ml with 1 M HCL.

TUBE B

(19) Wash 1 time with 5 ml diethyl ether.

(20) Remove excess ether with aspirator and boil off
remaining ether in hot water bath.

(21) Cool.

(22) Add 2 drops phenolphthalein solution.

(23) Add 1 N HaOH dropwise until solution turns red.

(24) Add 2 ml pH 8.5 carbonate buffer solution.

(25) Mix well.

(26) Add 5 drops methyl chloroformate solution.

(27) Mix vigorously.

(28) . Let stand for 8 min with occasional mixing.

(29) After 8 min add 0.75 ml reagent grade HCL.

(30) Mix thoroughly

(31) Wash 4 times with 5 ml diethyl ether. Removing
each time excess ether with the aspirator.

(32) Boil off excess ether in boiling water bath.

(33) Cool and make to 10 ml with deionized water.

(34) Read both tubes at 435 nm using water as a blank.

Subtract absorbance of Tube B from Tube A for results.

3. References

Booth, V.H. 1971. Problems in the determination of FDNB-available lysine. J. Sci. Food Agric. 22: 658.

Choi, R.P., A.F. Knocus, C.M. O'Malley and B.W. Fairbanks. 1949. A proposed method for the determination of color of dry products. J. Dairy Sci. 32: 589.

C. Effect of Protein Substitution on Non-Enzymatic Browning in

an Intermediate Moisture Food

Reprinted on the following pages is a copy of the article

submitted to the Journal of Food Science for publication. The paper

was presented at the 35th Annual IFT Meeting.

EFFECT OF PROTEIN SUBSTITUTION ON NON-ENZYMATIC

BROWNING IN AN INTERMEDIATE MOISTURE FOOD

R.A. Schnickels*, H.C. Warmbier** and T.P. Labuza

Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

* Present address: Burger King Corp., Minneapolis, MN

** Present address: American Can Co., Neenah, WS

1. Abstract

The effect of various proteins on the loss of lysine and
rate of non-enzymatic browning was studied in an intermediate moisture
food system containing glucose as 10% of the solids. It was found
that proteins which showed long induction times also had lower
browning rates, however, the rates did not correspond to loss of lysine
content. For almost every protein close to 50% of the lysine became
unavailable during the induction time before color development occurred.
Free lysine added to the formulation caused very rapid browning
suggesting rapid destruction.

2. Introduction

Non-enzymatic browning reactions occur very widely
during processing and storage of food materials. The colors produced
range from pale yellow to very dark brown, depending on the type of
food and/or the extent of the reaction. The non-enzymatic browning
reaction was first reported in 1912 by Maillard. A good review
was given by Hodge (1953) and still forms the basis of the study of
the reaction.

Basically, non-enzymatic browning takes place when
reducing sguars and proteins react in the presence of H_2O to form
brown pigments, resulting in the production of off-flavors (Markava,
1972) and loss of solubility and protein nutritional value (Rao and
Rao, 1972; Lea, 1958). This loss of protein nutritional value is
due to the fact that lysine, an essential amino acid is a primary
reactant. In particular, the N-terminal groups of peptides, proteins,
and ε-amino groups of lysine react to form nutritionally unavailable

compounds. Non-enzymatic browning is especially important in intermediate moisture foods because the amount of water present results in a much greater reaction rate than in dry food systems. In fact, Labuza (1971) showed that in relationship to water activity, the maximum rate of browning occurs in the intermediate moisture food region (a_w 0.60 to 0.85). This is because of the various effects of water, such as solubilization of reactants, and a decrease in viscosity of the absorbed aqueous phase. Because of the high reaction rate in this a_w region, one has a problem of using reducing sugars such as dextrose or corn syrup solids in food formulations, since they are primary reactants with the amino group.

Many intermediate moisture foods have been developed recently, such as complete breakfast replacements and high nutrition dietary bars. The intermediate moisture range was chosen for these products because of the good palatability while still maintaining the stability of the food towards microorganisms (Labuza, 1971). Unfortunately, even though microbial stability can be achieved, chemical degradation can not. For example, loss of protein nutritional value as discussed previously, rancidity and vitamin losses all can occur unless the proper additives or formulations are used (Chou et al., 1973; Lee and Labuza, 1975). It is quite evident that more information is needed on the stability of these foods.

The purpose of this study was to examine the effect of protein substitution in an intermediate moisture food model system on the rate of non-enzymatic browning. The supply and/or the cost of various proteins are such that food companies may find it necessary to

substitute their usual protein source with a different protein source. The problem lies in that the new protein may alter the stability of the finished product, specifically with respect to the rate of non-enzymatic browning during storage.

3. Materials and Methods

a. Model system

The proteins that were examined in this study were casein (technical grade - Coleman Bell, Inc), egg albumin (technical grade - Coleman Bell, Inc), whey protein (prepared at the University of Minnesota, spray dried, delactosed), fish protein concentrate (Bureau of Commercial Fisheries), spin textured soy protein (General Mills, Inc). The latter also was studied with 10% free lysine addition (weight/weight of protein). These proteins were substituted on a strict weight basis, thus the initial levels of available lysine (Table 2) and inherent reducing sugars would be different. However, this was done as it might be used commercially. The amount of residual reducing sugars in the protein should make no difference in the rate of browning as the amount of glucose added exceeds the 3:1 molar ratio with lysine as shown by Warmbier (1975). The composition of the dry model system is shown in Table 1.

The moisture content was determined on the "dry" mixed ingredients for each system by a GLC technique which was modified from the procedure described by Tjhio et al. (1969). Deionized water was then added to each system to achieve approximately 20% moisture in the final system. The final water activities were determined by the vapor pressure manometric technique (Karel and Labuza, 1967).

TABLE 1

COMPOSITION OF MODEL SYSTEM

Ingredient	%
K-sorbate	0.3
Glucose	10
Glycerol	20
Protein	30
Apiezon B oil	20
Microcrystalline cellulose	20
Water	as per Table 2

TABLE 2

CHARACTERISTICS OF MODEL SYSTEMS

Protein	Lysine content mg/100g solids	Moisture content of "dry" system g H_2O/100g solids	Moisture content of final system g H_2O/100g solids	Water Activity
Egg albumin	360	5.94	19.76	0.63
Casein	1416	9.24	18.34	0.70
Soy (TVP)	762	5.12	20.00	0.73
Wheat gluten	303	6.95	19.05	0.66
Fish protein concentrate	1283	7.53	18.25	0.68
Whey	857	1.85	18.53	0.78
Gluten + Lysine	3300	6.37	20.72	0.72

b. Storage of samples

The samples were sealed in 202 x 214 epoxy-lined cans, with the ends sealed with glyptal. The cans were stored at 35°C for 60 days and sampled periodically for non-enzymatic browning pigment production and loss of available lysine.

c. Browning determination

The Maillard browning reaction was monitored by measuring NEB (melanoidin) pigment production by a trypsin digest, aqueous extraction procedure of Choi et al. (1949) as modified by Labuza (1971). Available lysine content was determined by the FDNB method of Booth (1971). It should be noted that this latter method cannot be used when free lysine is present as in the lysine fortified wheat gluten system. This is because the free lysine left totally reacts and cannot be measured.

4. Results and Discussion

There are three important factors in non-enzymatic browning when considering using a protein in an intermediate moisture food. The first consideration is the induction time prior to visual detection of an increase in brown color. Second is the overall change in color due to the pigment production during the expected shelf-life of the product. The last consideration is the amount of available lysine lost.

The results of pigment production as a function of time are seen in Figure 1. The calculated rates and induction times are presented in Table 3. As seen, wheat gluten has the largest induction time and the smallest overall color change, while the wheat gluten with

NON-ENZYMATIC BROWNING PIGMENT PRODUCTION IN IMF SYSTEMS
AS A FUNCTION OF TIME

Non-Enzymatic Browning
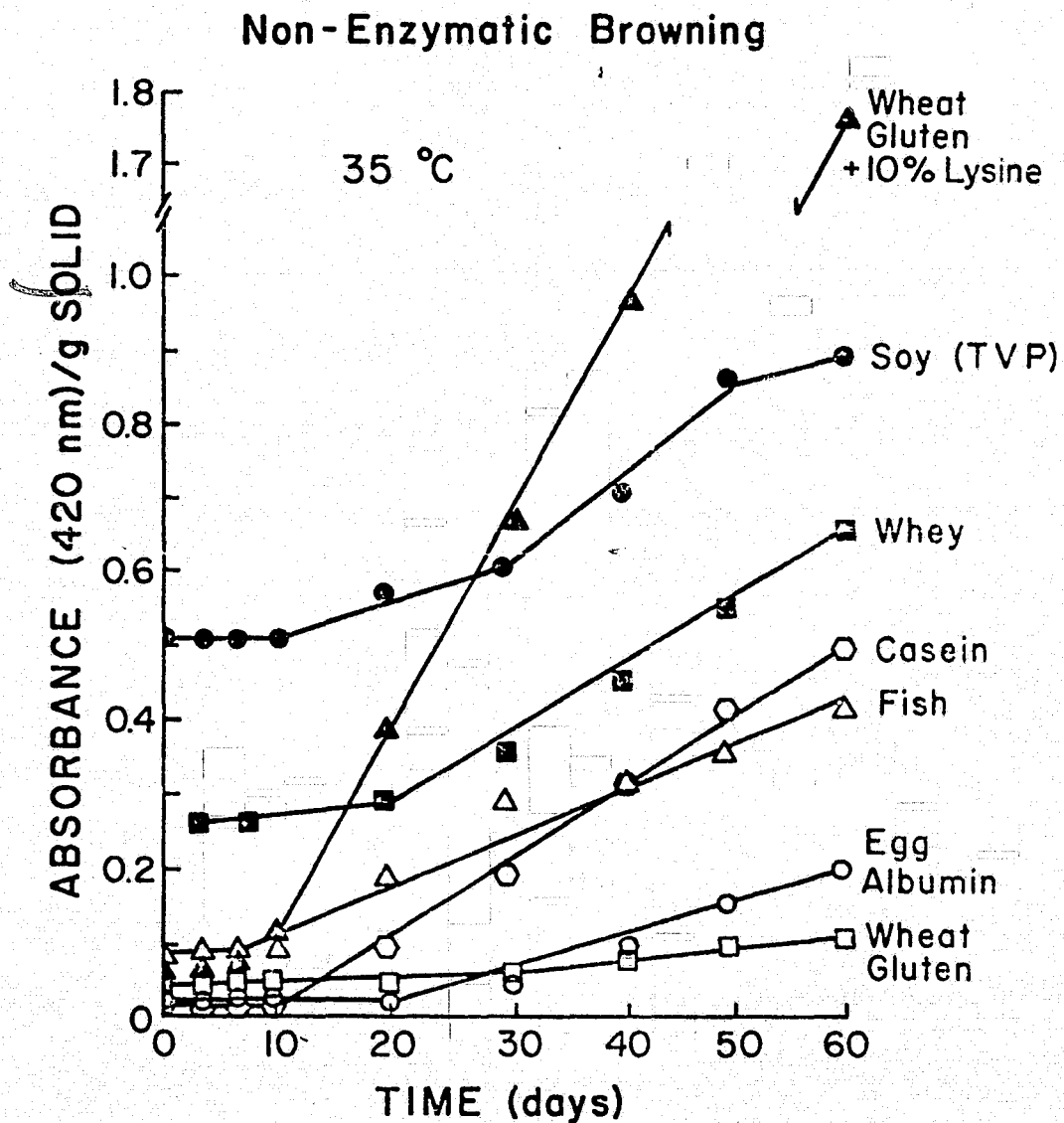
TABLE 3

NON-ENZYMATIC BROWNING RESULTS

Proteins	Induction Time (days)	Rate OD/day x 10^3
Wheat gluten + 10% free lysine	7	34.99
Casein	13	10.18
Whey	20	8.51
Soy (TVP)	20	7.45
Fish concentrate	10	5.93
Egg albumin	20	4.79
Wheat gluten	30	0.94

lysine fortification has the shortest induction time and the greatest

browning rate.   Figure 1 also shows the different initial colors due

to pre-processing.   For example, the textured soy protein system has

the highest initial color.   As noted in Table 3, there is some

correlation between the induction time and browning rate, with a lower

rate if the induction time is longer, except in the case of the fish

protein concentrate system.   Also, egg albumin reacts almost as

slowly as the other proteins even though there is low available

lysine in the initial sample.   This low value may have been due to

initial over-processing as generally one considers egg to be a high

quality protein.

Figure 2 shows the loss of available lysine in the model

systems.   As seen, the destruction of lysine occurs almost immediately.

In fact, the greatest loss of available lysine occurs within the first

30 days, with almost 50% lost prior to any visual appearance of brown

pigment.   This result indicates that brown pigment formation or the

lack of browning is not a direct indication of the extent of nutritional

losses.   The results, however, seem to follow the same pattern in

rate of loss as the browning results.

Table 4 shows the half lives and reaction rates for the

loss of available lysine.   A different pattern exists for lysine loss

than existed for non-enzymatic browning, with soy being the most rapid

for lysine loss as compared to casein being the most rapid for browning

of the non-fortified proteins.   Egg albumin for this test showed the

slowest rate.   With respect to browning, it was almost as fast as the

soy protein system.

FIGURE 2

DEGREE OF LOSS OF LYSINE IN IMF SYSTEMS
WITH DIFFERENT PROTEIN SOURCES
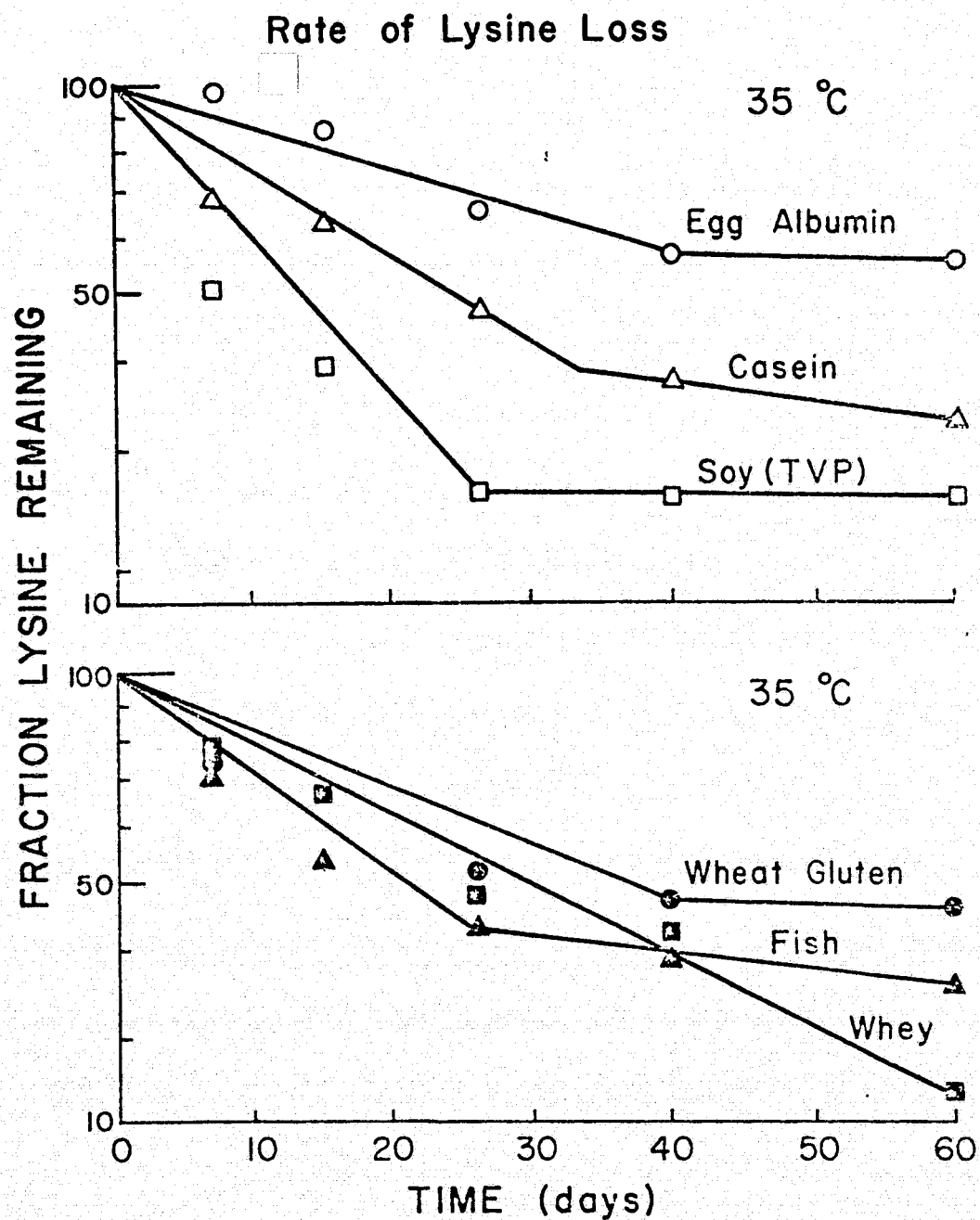
Rate of Lysine Loss

TABLE 4

RATE OF LYSINE LOSS IN THE SYSTEMS

Protein	$\Theta_{\frac{1}{2}}$ days (time for 50% destruction)	Rate* (kL_o)
Soy	11	52.6
Fish concentrate	19	53.8
Casein	21	38.7
Whey	25	21.3
Wheat gluten	29	8.2
Egg albumin	45	5.2

* g lysine/day x 10^3 per 100 g sample initially

Table 5 compares the rate of lysine loss in terms of half life with the browning induction times. These factors were used since they would be of importance to shelf life and processing. The proteins were ranked in order of the fastest rate to the slowest for each reaction. Also the initial lysine content is shown. A slight trend exists between the two reactions, but there is no pattern based strictly on lysine content. As seen, casein has double the lysine content of soy yet has half the lysine loss rate of soy. In contrast, casein has half the induction period of soy before browning occurs. The only protein comparable to casein is the fish protein concentrate.

This study shows that protein substitution in formulated IMF products is a complicated situation with respect to chemical and nutritional stability. The processor can substitute with a protein product of lower lysine content but this does not ensure either increased stability to browning or retention of the lysine content. In addition, this work shows significantly that supplementation with free lysine in an IMF product which contains reducing sugars cannot be done. Lastly this work points out that a quite large fraction of available lysine is lost even during the induction period. Examination of Table 5 shows that for almost every protein almost 50% of the lysine is destroyed within to slightly over the induction time.

5. References

Booth, V.H. 1971. Problems in determination of FDNB-available lysine. J. Sci. Food Agric. 22 (12): 658.

Choi, R.P., A.F. Koncus, C.M. O'Malley and B.W. Fairbanks. 1949. A proposed method for the determination of color of dry products of milk. J. Dairy Sci. 32: 580.

TABLE 5

COMPARISON OF LYSINE LOSS RATE vs. NEB FOR

THE VARIOUS PROTEIN SOURCES

Protein	Lysine content mg/100g solids	Lysine half life, $\theta_{\frac{1}{2}}$ (days)
Soy	762	11
Fish protein concentrate	1283	19
Casein	1416	21
Whey	857	25
Wheat gluten	303	29
Egg albumin	360	45

Protein	NEB Induction Time (days)
Fish protein concentrate	10
Casein	13
Soy	20
Whey	20
Egg albumin	20
Wheat gluten	30

Chou, H.E., K. Acott and T.P. Labuza. 1973. Sorption hysteresis and chemical reactivity: Lipid oxidation. J. Food Sci. 38: 316.

Hodge, J.E. 1953. Chemistry of browning reactions in model systems. Agric. & Food Chem. 1(15): 928.

Karel, M. and T.P. Labuza. 1967. Mechanisms of deterioration and formulation of space diets. Projects #41-609-66-46, Aerospace Medical Division, USAF 196601967, AF Contract #49(609)-2981.

Labuza, T.P. 1970. Properties of water as related to the keeping quality of foods. Paper presented at 3rd Int'l Congress of Food Science and Technology, Washington, D.C. Aug. 19-24, 1970. pp. 618-635.

Labuza, T.P. 1971. Analysis of storage stability of intermediate moisture foods. Contract NAS 9-10658. National Aeronautics and Space Administration, Food and Nutrition Office, Houston, Texas.

Lea, C.H. 1958. Chemical changes in the preparation and storage of dehydrated foods. In Fundamental Aspects of Dehydration and Storage of Dehydrated Foods. Soc. Chem. Ind., London. pg. 178.

Lee, S.H. and T.P. Labuza. 1975. Destruction of ascorbic acid as a function of water activity. J. Food Sci. 40: 370.

Maillard, L.C. 1912. Action of amino acids on sugars. Compt. Rend. 154: 66.

Markova, J., E. Honischova and J. Hampl. 19 . Non-enzymatic reactions in cereal production. Scientific Papers of Institute of Chem. Techn. Food. Prague, E-33, Czechoslovakia.

Rao, N.H. and M.M. Rao. 1972. Effect of non-enzymatic browning on the nutritive value of casein-sugar complexes. J. Food Sci. & Technol. (Mysore) 9(2): 66.

Tjhio, K., T.P. Labuza and M. Karel. 1969. Effects of humidification on catalysts and antioxidants in model systems. J.O.A.C.S. 46: 577.

Warmbier, H.C. 1975. Non-enzymatic browning kinetics of an intermediate moisture model food system. Ph.D. Thesis. University of Minnesota. St. Paul.

D.   Effect of Glycerol on Non-Enzymatic Browning in a Solid

Intermediate Moisture Model Food System

Reprinted on the following pages is a copy of the article

submitted to the <u>Journal</u> <u>of</u> <u>Food</u> <u>Science</u> for publication.   The paper

was presented at the 35th Annual IFT Meeting.

EFFECT OF GLYCEROL ON NON-ENZYMATIC BROWNING IN A

SOLID INTERMEDIATE MOISTURE MODEL FOOD SYSTEM

H.C. Warmbier*, R.A. Schnickels and T.P. Labuza

Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

* Present address: American Can Company, Neenah Technical Center,
333 No. Commercial St., Neenah, WI 54956

1. Abstract

Maillard browning is one of the main chemical reactions causing deterioration and shortening shelf-life of intermediate moisture food (IMF) systems. The purpose of this research was to study Maillard browning in an IMF model system containing casein, glucose and the liquid humectant glycerol. The kinetics of melanoidin pigment production, glucose utilization and loss of DNP-available lysine were studied as a function of temperature, moisture content and water activity. It was found that the factors which control reactant (glucose and available lysine) utilization also control end-product (melanoidin pigment) accumulation. The rate of the Maillard browning pigment production, after an initial induction period, follows zero order kinetics. The initial loss rate of both glucose and available lysine, however, follows first order kinetics. Exceedingly large nutritional (available lysine) losses occur before brown discoloration is appreciable. Slightly greater than one mole of glucose reacts per mold of lysine made unavailable. Based on this, nutritional losses may be relatively easily estimated by monitoring the loss of specific reducing sugars. The Maillard browning reaction proceeds 33 times faster at 45°C than at 25°C, with the maximum rate occurring at 0.4—0.5 a_w. This is an a_w range considerably lower than the 0.65—0.70 a_w range that is usually found for maximum browning in dehydrated foods. The reason for this downward shift in the a_w maximum is based on the fact that glycerol increases reactant mobility and/or solubility at a_w's below which water alone has a minimal effect.

2. Introduction

Food stability has been shown to be controlled by the moisture content or water activity (a_w) of foods (Bone, 1969; Labuza, 1970, 1975; and Lea, 1958). Bacterial degradation of foods can usually be controlled by keeping the water activity of the food at less than a_w 0.9. Yeast and mold growth can be inhibited by maintaining the a_w at less than 0.8 (Bone, 1969). Chemical degradation reactions of foods can usually be controlled by keeping the water activity or moisture content of foods low. Maximum stability is usually thought to occur when the food's moisture content is near the BET (Brunauer et al., 1938) monomolecular moisture layer coverage of foods (Salwin, 1959). As the moisture content increases above the BET coverage, the rate of chemical degradation of foods usually increases. And as the moisture content decreases below the BET coverage, lipid oxidation can again increase to cause rapid degradation of foods.

Water controls the degradative reactions of foods by various means. Osmotic shock, insufficient availability of required nutrients, or a build-up of metabolic end-products that are toxic to the organism are thought to be some of the means by which low moisture contents or a_w limits or inhibits the growth of microorganisms. Likewise, the chemical degradative reactions within foods are controlled through the effect of moisture content or a_w control on reactant dissolution, mobility and concentration, and reaction inhibition by mass action. An increase in moisture content or a_w can increase solute (reactant) solubility and/or mobility and thereby cause the rate of a chemical

0.60 to 0.85 (Karel and Labuza, 1969; Labuza, 1970; and Loncin et al., 1968).

Very little data is available on the extent of Maillard browning in IMF systems to which a liquid humectant has been added. A liquid humectant can increase the palatability of a food or lower the water activity of the food and thereby increase its microbial stability. Eichner and Karel (1972) studied the extent of Maillard browning in a liquid model system of glycine-glucose which contained the humectant glycerol. They found that under certain conditions the maximum in browning, as measured by melanoidin pigment production, occurred near a_w 0.4. They concluded that glycerol, through its plasticizing effect, partially restores reactant mobility to increase the browning rate at low moisture contents.

Since solid IMF items, such as meal replacement items, are becoming more common in the marketplace, a study of the rate of the Maillard browning reaction as well as a determination of the loss of nutritional value within these IM foods is necessary. This current study examines the rate of Maillard browning as it occurs during the storage of a solid IMF model system which contains the liquid humectant glycerol.

3. Materials and Methods

To facilitate the collection of browning data, a model food system was used. The composition of the model system is shown in Table 1. Potassium sorbate was used as an antimicrobial agent. Glucose is the sole source of reducing sugar for the Maillard reaction in this study. Glycerol is used as a liquid humectant to control the

TABLE 1

MODEL SYSTEM COMPOSITION

Component	Grams
K-sorbate	0.3
Glucose	10.0
Glycerol	20.0
Casein	30.0
Apiezon B Oil	20.0
Microcrystalline cellulose	20.0
Water	Variable

water activity and plasticity of the model system. Casein serves as the only source of free amino groups for the Maillard reaction. Apiezon B oil, which is liquid at room temperature, adds plasticity to the product. It is saturated and therefore should not participate in the Maillard reaction. Microcrystalline cellulose is inert to the Maillard reaction and serves as a solid support for the model system. Water is added in varying amounts so the rate of Maillard browning could thereby be studied as a function of water activity or moisture content. The average initial glucose/available lysine molar ratio is 2.9.

The components, as tabulated in Table 1, were mixed together in descending order of appearance in the Table. Water was added by two different methods such that the effect of method of water addition on the rate of Maillard browning could be studied. Direct Mix systems were brought to proper moisture content by mixing in a predetermined amount of liquid water to the non-aqueous ingredients. The Direct Mix samples were then held in vacuo over an appropriate saturated salt solution (Rockland, 1960) for one day at room temperature for final moisture equilibration. The Humidified Mix samples were humidified by storing the mixed non-aqueous components in vacuo over an appropriate saturated salt solution for three to six days at room temperature until the proper water activity was obtained. Moisture content was measured with a methanol extraction GC technique (Tjhio et al., 1969). Water activity was measured by a vapor pressure manometric technique (Karel and Labuza, 1967; Karel and Nickerson, 1964).

Following the formulation process, including the addition of water, the samples were transferred to 202 x 214 epoxy-lined cans. The cans were sealed and the newly sealed ends were dipped in glyptol to retard moisture loss should the can seal not be perfect. The canned samples were then incubated at 25, 35 or 45°C and periodically analyzed for extent of Maillard browning. If sample analysis could not be done on the desired day, the samples were held at -29°C until the analysis could be performed.

The Maillard browning reaction was monitored by measuring NEB (melanoidin) pigment production, glucose utilization, and loss of available lysine. NEB pigment concentration was measured by the trypsin digest, aqueous extract procedure of Choi et al. (1949) as modified by Labuza (1971). Glucose content was measured with a glucose oxidase Blood Sugar test kit (Bolhringer Mannheim Corp., New York, Cat. No. 15756. Method: adapted from Werner, W., H.G. Rey and Hl Wielinger. 1970. Z. Anal. Chem. 252: 224). The FDNB method of Booth (1971) was used to measure available lysine content.

4. Results and Discussion

The increase of melanoidin pigment concentration as a function of storage time, temperature and a_w is presented in Figure 1. After an initial induction period, the amount of NEB pigment increases linearly with time for each condition. During the initial induction period, predominantly colorless browning intermediates are being formed. After a sufficient amount of these intermediates have been formed, the rate of melanoidin pigment production follows zero order kinetics.

FIGURE 1

NEB PIGMENT PRODUCTION IN AN IMF MODEL SYSTEM AS
A FUNCTION OF WATER ACTIVITY AND TEMPERATURE
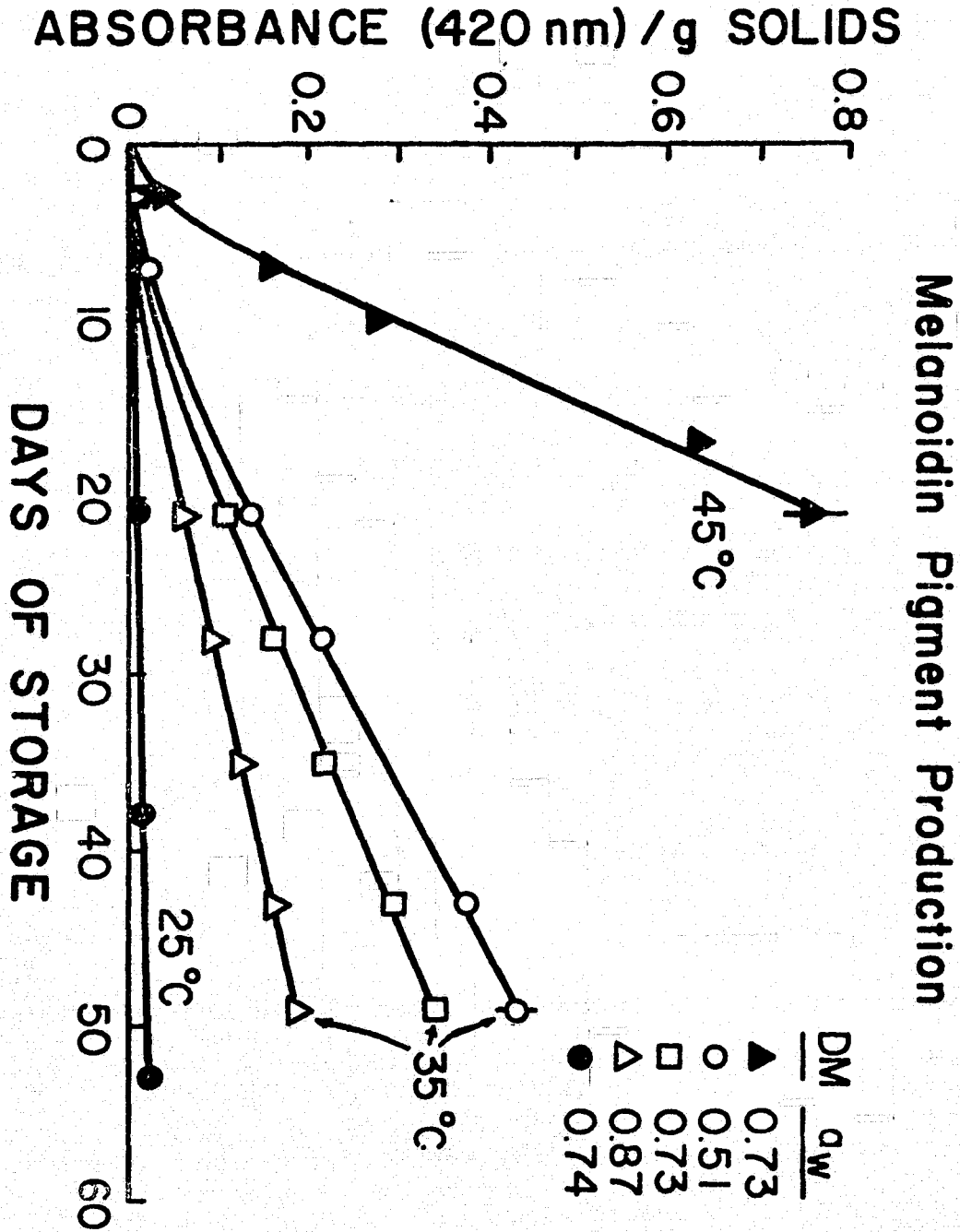
TABLE 2

PERCENT LOSS OF REACTANTS (GLUCOSE AND AVAILABLE LYSINE) DURING THE INDUCTION TIME FOR MELANOIDIN PIGMENT PRODUCTION

Temp. $^{\circ}$C	Avg. a_w	Browning Induction Time (Days)	Glucose % Loss	Lysine % Loss
25	0.86	100	10	60
25	0.72	100	21	65
25	0.67	100	26	55
35	0.87	20	13	20
35	0.51	20	33	60
35	0.32	20	18	12
45	0.84	7	29	37
45	0.39	7	33	69
45	0.14	7	17	71

Temperature controls both the length of the induction period and the rate of pigment production during the zero order period. At the higher temperature, the rate of NEB pigment production is increased. The average activation energy for pigment production is 32.9 Kcal/mole, which gives a Q_{10} of about 6.1 between 25 and 35°C and 5.4 between 35 and 45°C. Thus, the reaction occurs 33 times faster at 45°C than at 25°C. Also, as is shown in Table 2, the induction time is less at the higher temperature.

The 35°C data of Figure 1 also shows that browning rates are controlled by water activity. The influence of water activity on browning rate is further shown in Figure 2 for 35 and 45°C. The same pattern was also found at 25°C (Warmbier, 1975). As seen, the maximum rate of NEB pigment production occurs at a_w 0.45 to 0.55; this is unlike that found in most solid food systems which show a maximum rate near a_w 0.7—0.8. The rate maximum at 35°C occurs at a moisture content of 8.9 g H_2O/100g solids. At 45°C, the maximum is at 10 g H_2O/100g solids. The calculated BET monolayer for the systems of this study is 8.1 g H_2O/100g solids. A dehydrated food is usually considered to be most stable to chemical reactions if its moisture content is at or near the BET monolayer (Salwin, 1959). In this study, however, with a liquid glycol added a maximum in the rate of degradation occurs close to the BET value.

The above observation is similar to the findings of Eichner and Karel (1972). Their liquid model system containing glycerol had a maximum in browning at a_w 0.41 when stored at 37°C. They concluded that glycerol can increase the rate of browning at low a_w

Rate of Melanoidin Pigment Production

35 C Storage

45 C Storage

● DM
■ HM
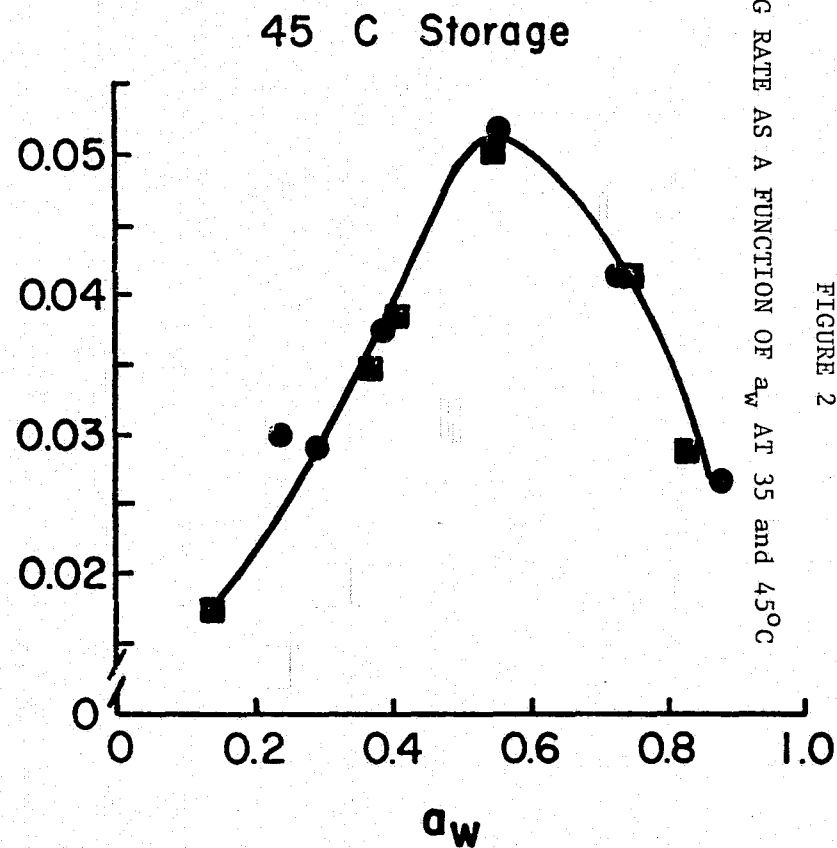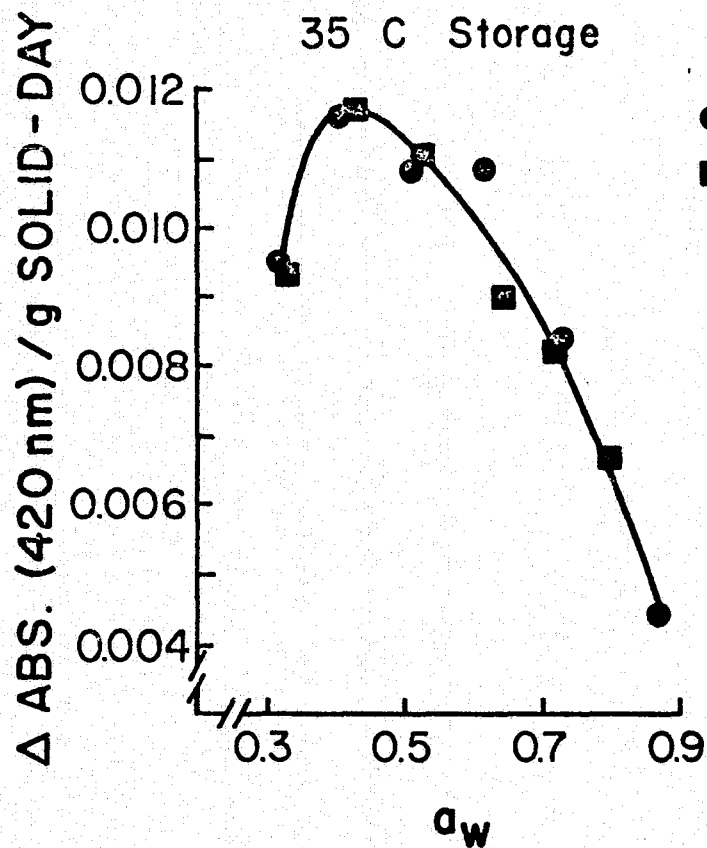
BROWNING RATE AS A FUNCTION OF a_w AT 35 and 45°C

FIGURE 2

-157-

GLUCOSE LOSS - 45 °C

LYSINE LOSS - 45 °C

FRACTION REMAINING

1.0

0.5

0.1

DM	a_w
▲	0.88
■	0.73
●	0.56

DM	a_w
▲	0.85
■	0.78
●	0.57

1.0

0.5

0

0   10   20   30

0   10   20

TIME (days)
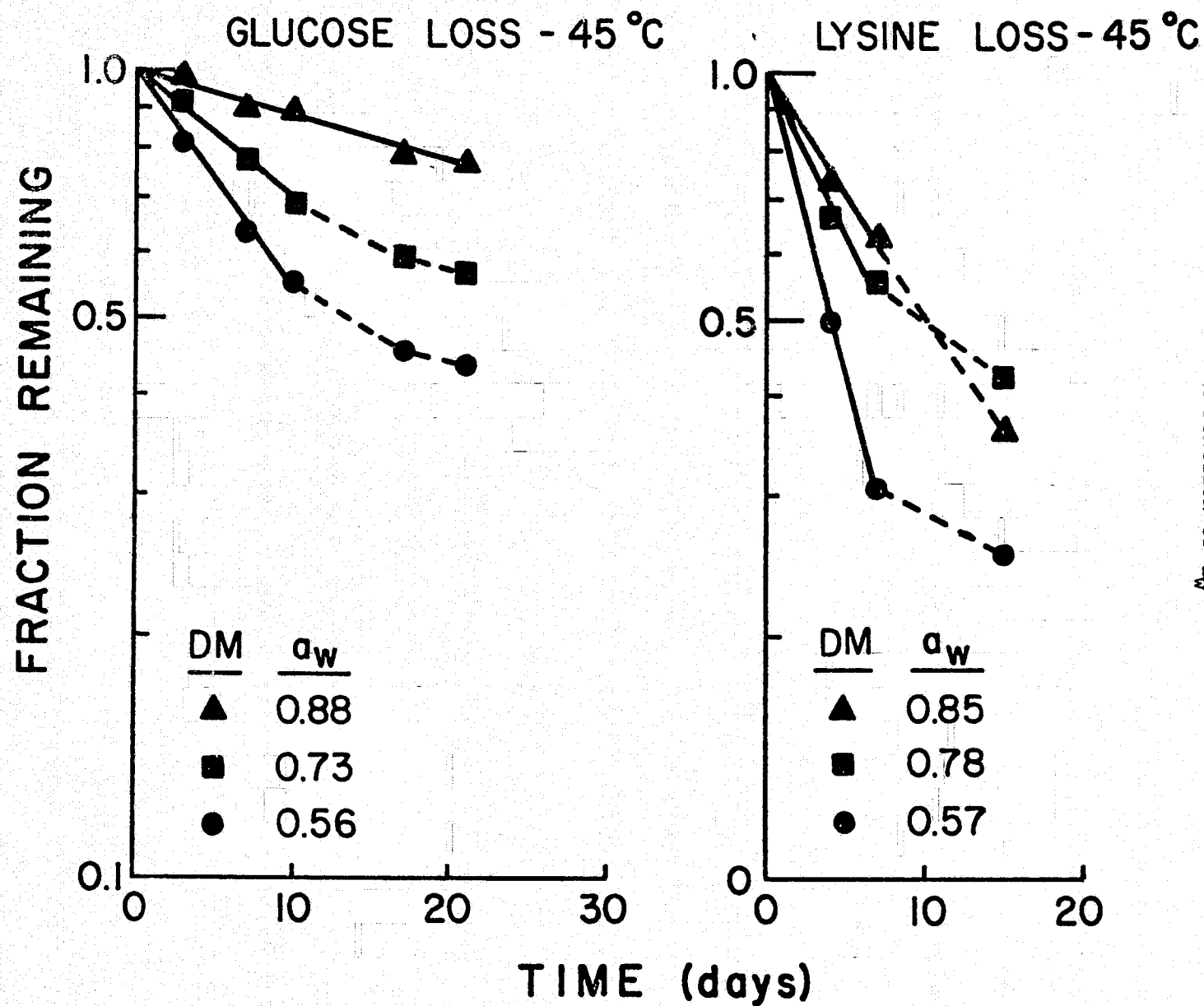
LOSS OF GLUCOSE AND LYSINE IN AN IMF MODEL SYSTEM
AS A FUNCTION OF a_w

FIGURE 3

values by increasing reactant mobility and that as a_w increases, the water decreases the browning rate by a mass action effect. This should also be the mechanism in the solid food systems used in this current study. The results of Figure 2 thus indicate that glycerol could cause a decrease in browning if it is added to intermediate moisture foods.

One additional observation to be drawn from Figure 2 is that the method of water addition to the food samples does not appreciably influence the rate of NEB pigment production. At any given water activity or moisture content, samples to which liquid water was added directly (Direct Mix system) browned at the same rate as those samples to which water was added by a vapor humidification process (Humidified Mix system). This suggests that solubilization of the reactants is the same no matter what the method of addition of the water is.

As stated earlier, the induction period must occur before the rate of melanoidin pigment production follows (constant) zero order kinetics. During this initial storage period when melanoidin pigment production is not yet appreciable, reactants of the Maillard reaction are utilized to form colorless browning intermediates. Figure 3 shows the loss of both glucose, a reducing sugar, and available lysine at 45°C. The lysine serves as a source of free ε-amino groups for the formation of glycosylamines for the Maillard reaction. The data indicate that initially the destruction rates of both glucose and available lysine follow first order kinetics. A significant amount of glucose and available lysine is destroyed even before melanoidin

pigment production becomes appreciable. As is shown in Table 2,
up to one-third of the glucose and, more importantly, as much as
70% of the available lysine is reacted before the rate of melanoidin
pigment follows zero order kinetics and visual browning appears.
Obviously this has serious nutritional implications. A food in which
Maillard browning can occur may not have produced melanoidin pigments
at a sufficient rate such that the color of the food becomes
objectionably brown during processing or storage. However, the
protein nutritional loss within the food, as indicated by decreased
available lysine content, may be significant.

As previously indicated in Figure 3, water influences
the rate of glucose and available lysine loss. Figures 4 and 5 show
the initial (first order) loss rate of glucose and available lysine at
35 and 45°C as a function of water activity. In general, the loss
rate of both glucose and available lysine are controlled in the same
way by water activity. It can also be observed that the maximum
rate of these reactants occurs near the same a_w as for the maximum
rate of browning.

Loncin et al. (1968) showed that maximum lysine loss in
dehydrated foods occurs at an a_w greater than approximately a_w 0.6.
Their food systems were very similar to our model systems except a
liquid humectant was not added to their systems. Therefore, the
downward shift in the a_w maximum for lysine loss and glucose loss must
be caused by glycerol in a manner similar to that which controls
NEB pigment production as proposed by Eichner and Karel (1972). The
practical significance of the addition of glycerol is thus obvious.

FIGURE 4

INITIAL GLUCOSE AND LYSINE LOSS RATES
AS A FUNCTION OF a_w AT 45°C
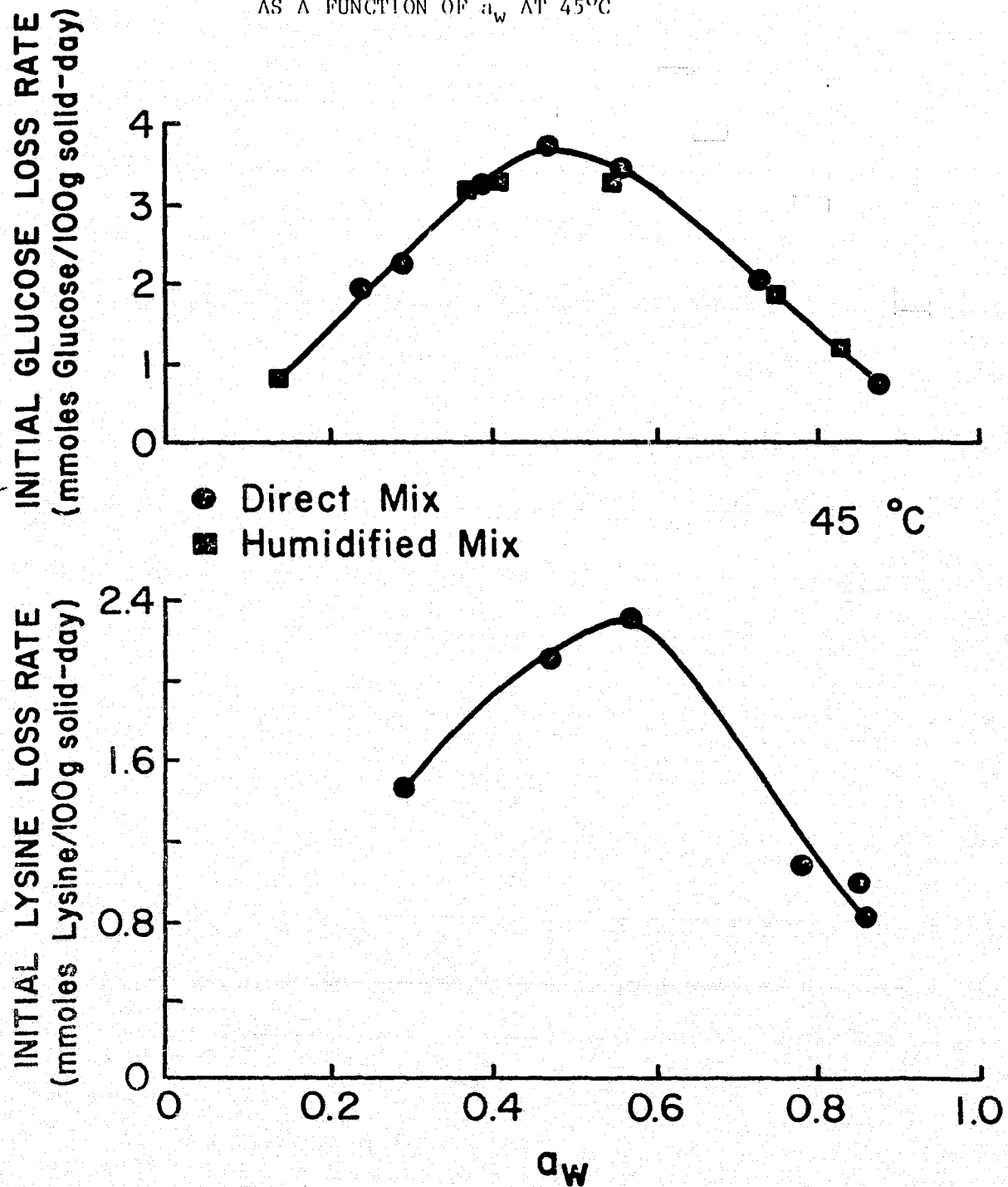
FIGURE 5

INITIAL GLUCOSE AND LYSINE LOSS RATES
AS A FUNCTION OF a_w AT 35°C
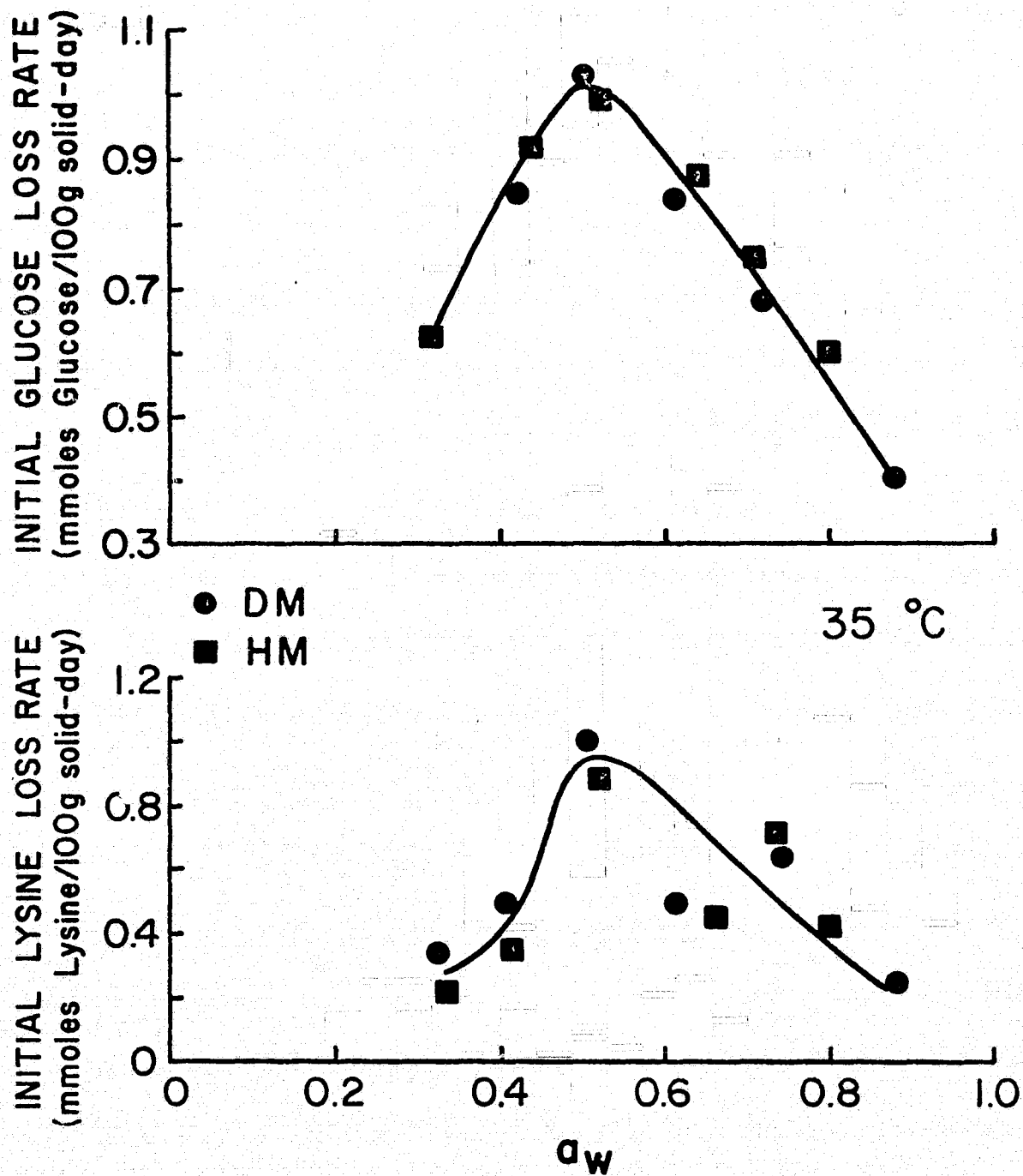
Glycerol, or perhaps other liquid humectants, when added to meal bars, semi-moist pet foods, or other IMF's, can decrease the rate at which lysine, and perhaps other essential nutrients with free amino groups, becomes unavailable.

One further observation drawn from this study is that at 35 and 45°C, an average of 1,3 moles of glucose initially react for one mole of lysine that becomes unavailable. From a practical standpoint, the monitoring of the loss of glucose or other specific reducing sugars may be used as a relatively rapid and easy method for estimating the nutritional (available lysine) loss of foods suspected of being degraded by the Maillard non-enzymatic browning reaction. This type of method is much more simple than the FDNB procedure which requires much technical training and over 24 hr for the determination of available lysine.

5. Summary

Three significant and practical conclusions can be found from this research. First, the addition of glycerol to inter-mediate moisture foods can cause the a_w maximum for the Maillard reaction to be shifted downward. Thus, IMF's which contain glycerol, or perhaps other liquid humectants, would have a browning rate that is less than would occur if a liquid humectant were not present. Second, the Maillard reaction can cause significant nutritional losses, e.g. of available lysine, to occur before the food has become appreciably or objectionably brown. Third, the protein nutritional loss of foods susceptible to Maillard browning may be relatively easily and quickly estimated by following specific reducing sugar loss during storage.

6. References

Bone, D. 1969. Water activity: Its chemistry and application. Food Prod. Development. August. pg. 81.

Booth, V.H. 1971. Problems in the determination of FDNB-available lysine. J. Sci. Food Agric. 22(12): 658.

Brunauer, S., P.H. Emmett and E. Teller. 1938. Adsorption of gases in multimolecular layers. J. Amer. Chem. Soc. 60: 309.

Choi, R.P., A.F. Koncus, C.M. O'Malley and B.W. Fairbanks. 1949. A proposed method for the determination of color of dry products of milk. J. Dairy Sci. 32: 580.

Eichner, K. and M. Karel. 1972. The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. J. Agric. Food Chem. 20(2): 218.

Karel, M. and T.P. Labuza. 1967. Mechanisms of deterioration and formulation of space diets. Project #41-609-66-46. Aerospace Medical Div. USAF 196601967. AF Contract 49-(609)-2981.

Karel, M. and T.P. Labuza. 1969. Optimization of protective packaging of space foods. Final Report. USAF School of Aerospace Medicine. Brooks Air Force Base, Texas. Contract #F 41-609-68-C-0015.

Karel, M. and J.T.R. Nickerson. 1964. Effects of relative humidity, air, and vacuum on browning of dehydrated orange juice. Food Technol. 18: 104.

Labuza, T.P. 1971. Properties of water as related to the keeping quality of foods. Proc. of Third Int'l Congress of Food Science and Technology. Washington, D.C. August 9-14, 1970. pp. 618-635.

Labuza, T.P. 1971. Analysis of storage stability of intermediate moisture foods. Contract NAS 9-10658. National Aeronautics and Space Administration, Food and Nutrition Office, Houston, Texas.

Labuza, T.P. 1975. Oxidative change in foods at low and intermediate moisture levels. In Water in Foods. R. Duckworth, editor. Academic Press, London.

Lea, C.H. 1958. Chemical changes in the preparation and storage of dehydrated foods. In Fundamental Aspects of Dehydration and Storage of Dehydrated Foods. Soc. Chem. Ind. London. pp. 178-196.

Loncin, M., J.J. Bimbenet and J. Lenges. 1968. Influence of the activity of water on the spoilage of foodstuffs. Food Technol. 3: 131.

Rockland, L.B. 1960. Saturated salt solutions for solute control of relative humidity between 5 and 40°C. Anal. Chem. 32: 1375.

Rockland, L.B.   1969.   Water activity and storage stability.   Food
    Technol.   23: 1241.

Salwin, H.   1959.   Defining minimum moisture contents for dehydrated
    foods.   Food Technol.   13: 594.

Tjhio, K., T.P. Labuza and M. Karel.   1969.   Effects of humidification
    on catalysts and antioxidants in model systems.   JOACS 46: 577.

Warmbier, H.C.   1975.   Non-enzymatic browning kinetics of an intermediate
    moisture model food system.   Ph.D. Thesis.   University of
    Minnesota.   St. Paul

E.   Effect of Food Humectants on Non-Enzymatic Browning

1.   Introduction

Non-enzymatic browning is one of the major deteriorative reactions occurring in the semi-moist foods, especially in the water activity range of 0.60 to 0.85. It leads to off-colors, off-flavors and loss of nutritional value. The rate of browning is usually nearly negligible at the monolayer moisture content and rises to a maximum in the 0.60—0.85 a_w range, decreasing again at the high a_w's. The mechanisms of action of water are:

a.   Solubilization and dissolution of the reacting solutes — reducing sugars and amines.

b.   Increased moisture decreases aqueous phase viscosity and thus increases reaction rate.

c.   Above a certain moisture level any increases in water content decreases the reactant concentrations and thus decreases reaction rate after reaching a maximum.

d.   Since 3 moles of water are produced per mole of reducing sugar, water decreases the rate through mass action.

Because of this problem, processors have been searching for means to prevent the reaction. Raising the moisture content and thus the a_w to above 0.85 lowers the rate but makes the product highly susceptible to microbial deterioration. Reducing the moisture leads to a dry, unpalatable product. The standard sulfite or SO_2 additive is not allowed for use except with dry fruit and vegetable products. A non-toxic browning inhibitor would be highly useful.

The recent study on non-enzymatic browning in an intermediate moisture model food system presented in section D showed a maximum browning rate at a_w 0.43, somewhat lower than the a_w range of 0.65—0.70 found to give maximum browning levels in many foods. This system contained the humectant glycerol. This finding is of considerable interest in relation to the importance of browning as a principal cause of deterioration in intermediate moisture foods and the fact that no good inhibitor systems exist. It is possible that the glycol may supply this inhibition.

In light of previous results found regarding the effect of glycerol on non-enzymatic browning, the following study was prepared to:

a. Determine the rate of non-enzymatic browning in a casein-glucose model system without the addition of glycerol, and to determine the a_w at which maximum NEB occurs during storage for up to 60 days at $35^\circ C$.

b. Compare the humectants glycerol, sorbitol, propylene glycol and 1,3 butylene glycol in terms of development of NEB in an IMF model system.

2. Materials and Methods

a. Model system preparation

The model system used in these studies (Table 1) was based on that described by Labuza (1974), except that Apiezon B oil was replaced by silicone oil (Cat. No. 6428-R15, Arthur H. Thomas Co., Philadelphia, Pa., 19105) due to the unavailability of the former.

Components were added in descending order as listed

in Table 1. Potassium sorbate pellets were ground to a fine powder and mixed in a beaker with lump free powdered glucose. The humectant was dispersed uniformly and casein added, followed by silicone oil. Freeze-dried microcrystalline cellulose is mixed uniformly into the system, followed by sufficient water (0 to 30 or 40 g H_2O/100g non-aqueous solids) to produce a_w levels of approximately 0.2—0.9. Samples containing no added water were stored on petri dishes over drierite or a saturated solution of LiCl (a_w 0.11, 20°C) for sufficient time to produce a_w levels in the range 0.25 to 0.20.

The mixed components were stored in beakers covered with parafilm for 1-2 days at room temperature for equilibration (except for samples stored over drierite or saturated salt solutions), 20-40g samples were sealed into 202 x 214 epoxy-lined tin cans, the ends were dipped into glyptol to prevent moisture transfer, and the cans stored at 35°C for up to 60 days. Samples were analyzed for moisture content, a_w, extent of non-enzymatic browning and glucose retention.

b. Determination of moisture content

(1) Extract 3 g samples (weighed accurately, in duplicate) with 20 ml anhydrous methanol in a 50 ml Erlenmeyer flask for 30 min at room temperature on a reciprocal shaker at 150 CPM.

(2) Determine moisture content from the ratio of water to methanol peak areas of known water-methanol mixtures (0-1g water/20 ml methanol) after gas chromatographic separation. Peak areas were printed by electronic integrator.

TABLE 1

MODEL SYSTEM COMPOSITION

Component	Grams
K-sorbate	0.3
Glucose	10.0
Humectant*	20.0
Casein	30.0
Silicone oil	20.0
Microcrystalline cellulose	20.0
Water	Variable

* Run 1   -   no humectant added

  Run 2   -   glycerol

  Run 3   -   1,3-butylene glycol

  Run 4   -   propylene glycol

  Run 5   -   sorbitol

(3) GLC separation conditions were:

Chromatograph: Hewlett-Packard Model 7620A

Column: Poropak Q (91.5 cm, 0.32 cm ID)

Column temperature: 110°C

Injector temperature: 270°C

Polarity: A

Helium flow rate: 1.75 (42 ml/min)

Bridge current: 130-140 ma.

Sample size: 2 μl

Integrator: Hewlett-Packard Model 3370B

Input selector: 2

Noise suppression: 2

Attenuation: 100

Slope sensitivity: up, 3; down, 1

Baseline reset: 0.3

Area threshold: 100 or 1000

Shoulder control: 1000

c. Determination of a_w

A_w was determined by the vapor pressure manometric

(VPM) technique as described by Labuza (1973).

d. Determination of non-enzymatic browning

A modification of the method of Choi et al. (1949)

was used:

(1) To 2.00g sample (duplicate) in a 50 ml Erlenmeyer

flask add 2.5 ml 10% (w/w) trypsin suspension and 20 ml phosphate

buffer (pH 7.8).

(2) Incubate system for 2 hr at 45°C on reciprocal shaker at 150 CPM.

(3) Following incubation, add 2 ml 50% (w/v) trichloroacetic acid and 1.0g celite and filter through Whatman #1 filter paper.

(4) Measure absorbance of sample at 420 nm against a reagent blank. If turbidity persists after filtration, measure absorbance also at 600 nm.

(5) Report browning as corrected absorbance at 420 nm/g solids.

e. Determination of residual glucose

Glucose content was measured with a glucose oxidase blood sugar test kit (Boehringer Mannheim Corp., New York. Catalogue No. 15756).

(1) To 3.00g sample (duplicate) in a 125 ml Erlenmeyer flask add 100g water and extract for 30 min on a reciprocal shaker at 180 CPM.

(2) Filter contents through Whatman #1 filter paper.

(3) To duplicate aliquots of 5.0 ml buffered glucose oxidase-heroxidase test solution 2 in a test tube add 10 µl filtrate with an Eppendorf micropipette and mix thoroughly in a vortex mixer.

(4) Incubate system for 25-50 min (usually 40 min) at room temperature out of direct sunlight.

(5) Measure absorbance at 600 nm of samples and test kit standard (containing 0.2 ml glucose test solution 1 and 5 ml

solution 2) against a reagent blank (0.2 ml water and 5 ml

solution 2).

     f. Calculate glucose content as g glucose/100g solids.

  3. Results and Discussion

     a. Moisture sorption isotherms of model system

The moisture sorption isotherms (determined at room

temperature, 20-25°C) for each run, are shown in Figure 1 and the

data and monolayer are in Table 2. As expected, systems with glycols

contain greater quantities of water than do systems without humectants

in the IMF region. This, however, does not hold true for systems

at low a_w, possibly due to blockage of hydrogen bonding sites. The

difference is very small. This is also evidenced by the monolayer

a_w's which are all fairly close.

     b. Non-enzymatic browning and glucose destruction

Tables 3 through 7 contain the results of the increase

in browning as a function of time. Figures 2 and 3 show representative

data that were plotted. The slope of the linear portion after the

induction period was measured and calculated as the rate of browning

both on a total solids and non-humectant solids basis. In all cases,

the induction period before the linear portion of the curve was very

short. For the slowly reacting samples the calculated rate includes

part of the induction time.

The calculated rates were plotted as a function of

a_w and moisture in Figure 4 on a total solids basis for the glycerol vs.

the control and for all the systems in Figure 5. As seen, the three

polyols follow the same type of curve as does the control, showing a

FIGURE 1
MOISTURE SORPTION ISOTHERMS IN MODEL SYSTEMS AT 35°C

FIGURE 1
MOISTURE SORPTION ISOTHERMS IN MODEL SYSTEMS AT 35°C
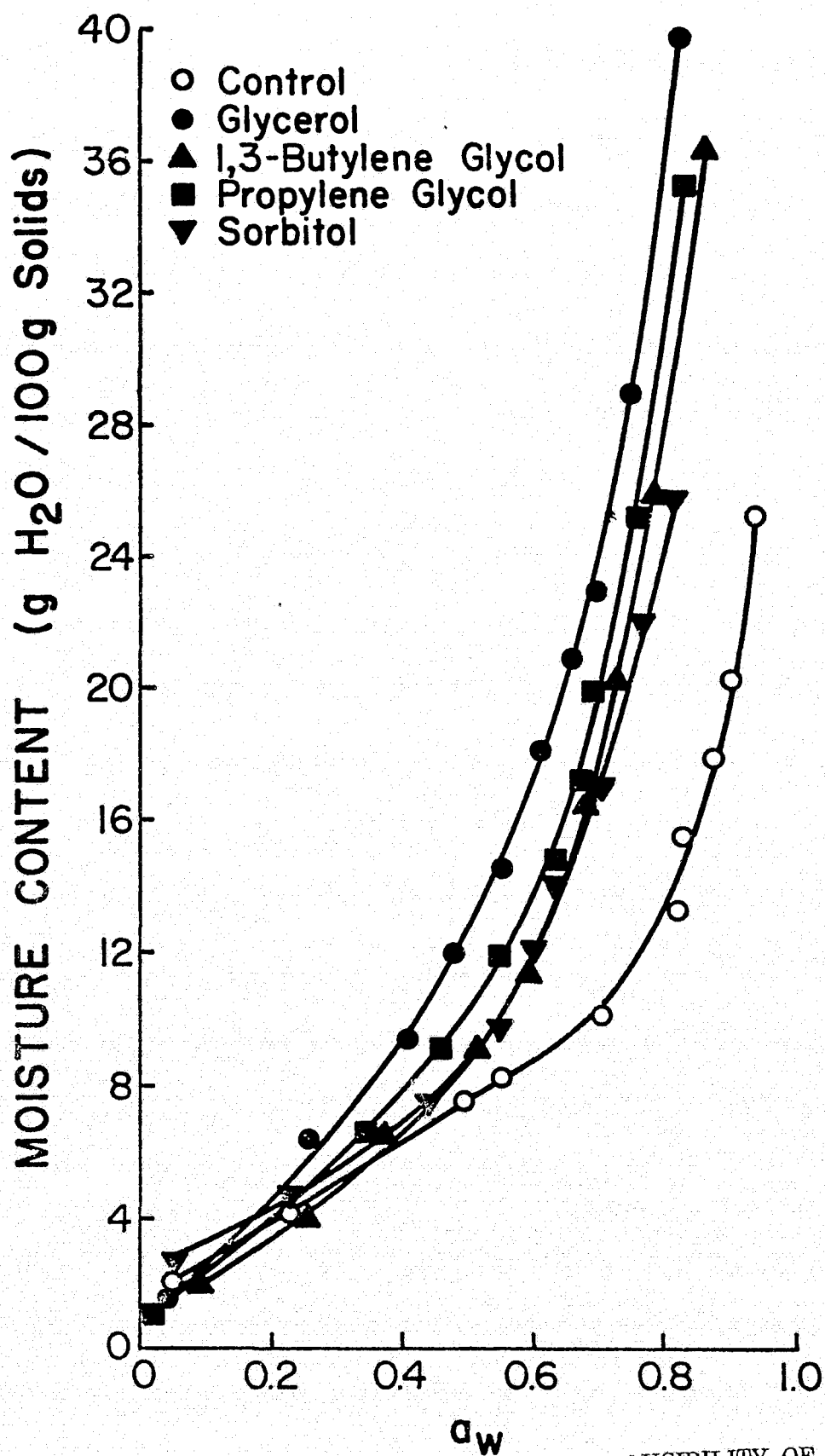
TABLE 2

MOISTURE - A_W DATA

Water activity		Moisture content (g H_2O/100g solids)			
	Control	Glycerol	Butanediol	Propylene glycol	Sorbitol
0.02				1.15	
0.04		1.74			
0.05	2.20				2.63
0.09			1.97		
0.23	4.42			4.14	4.65
0.26		6.42	4.09		
0.35				6.74	
0.37			6.49		
0.41		9.27			
0.46				9.37	7.31
0.49	7.68	12.04			
0.51			9.12		
0.55		14.73		11.91	9.65
0.60		18.20	11.42		12.06
0.64				14.74	14.03
0.66		21.05			
0.68			16.60		
0.70	10.29	23.41		19.99	16.99
0.73			20.42		
0.75		29.53	24.36		
0.78			26.02	25.18	19.90
0.82	13.61	39.80			25.65
0.83	15.88			35.35	
0.87	18.06		36.43		
0.90	20.58				
0.93	25.45				

monolayer:					
m_o	5.66	10.10	7.51	5.08	5.57
a_o	0.35	0.42	0.45	0.30	0.35

FIGURE 2

INCREASE IN NEB PIGMENT PRODUCTION AT 35°C AS A
FUNCTION OF a_w AND STORAGE TIME

Control System
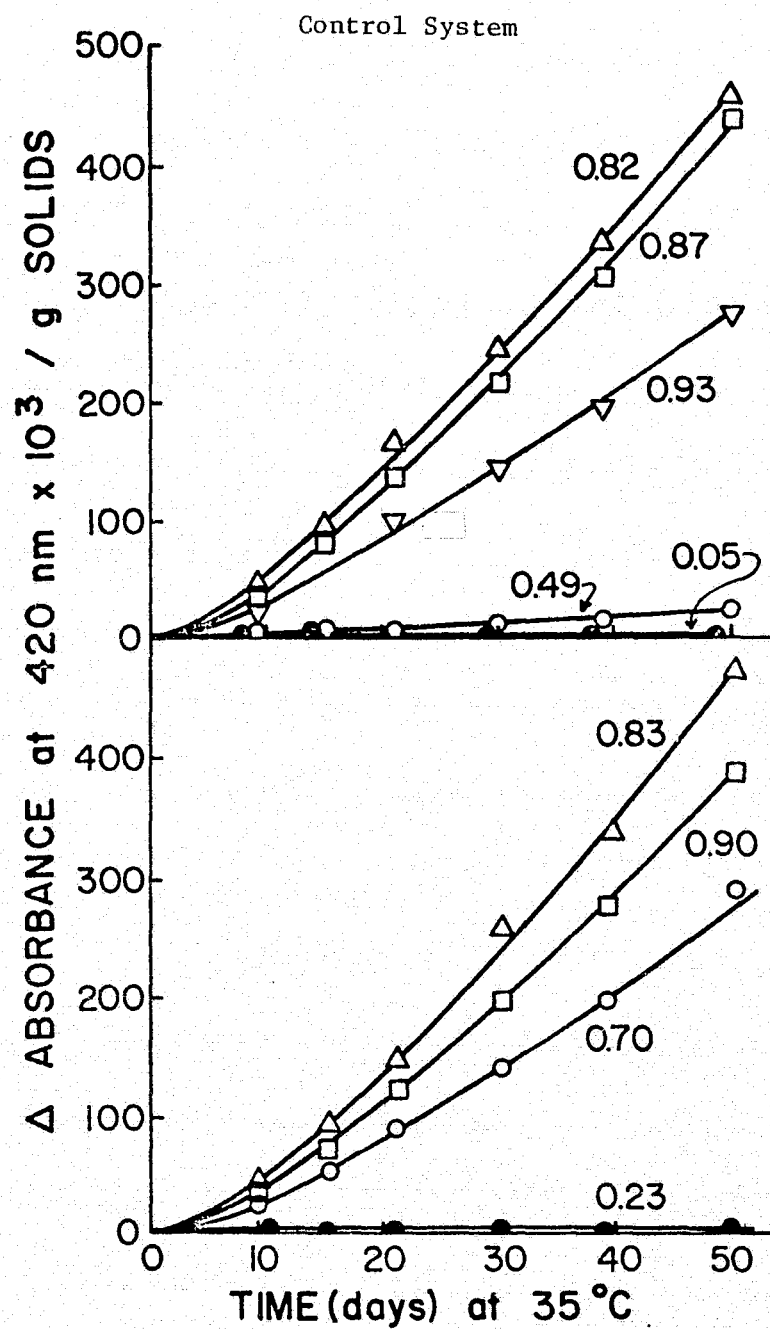
FIGURE 3

INCREASE IN NEB PIGMENT PRODUCTION AT 35°C AS A
FUNCTION OF a_w AND STORAGE TIME

Glycerol System
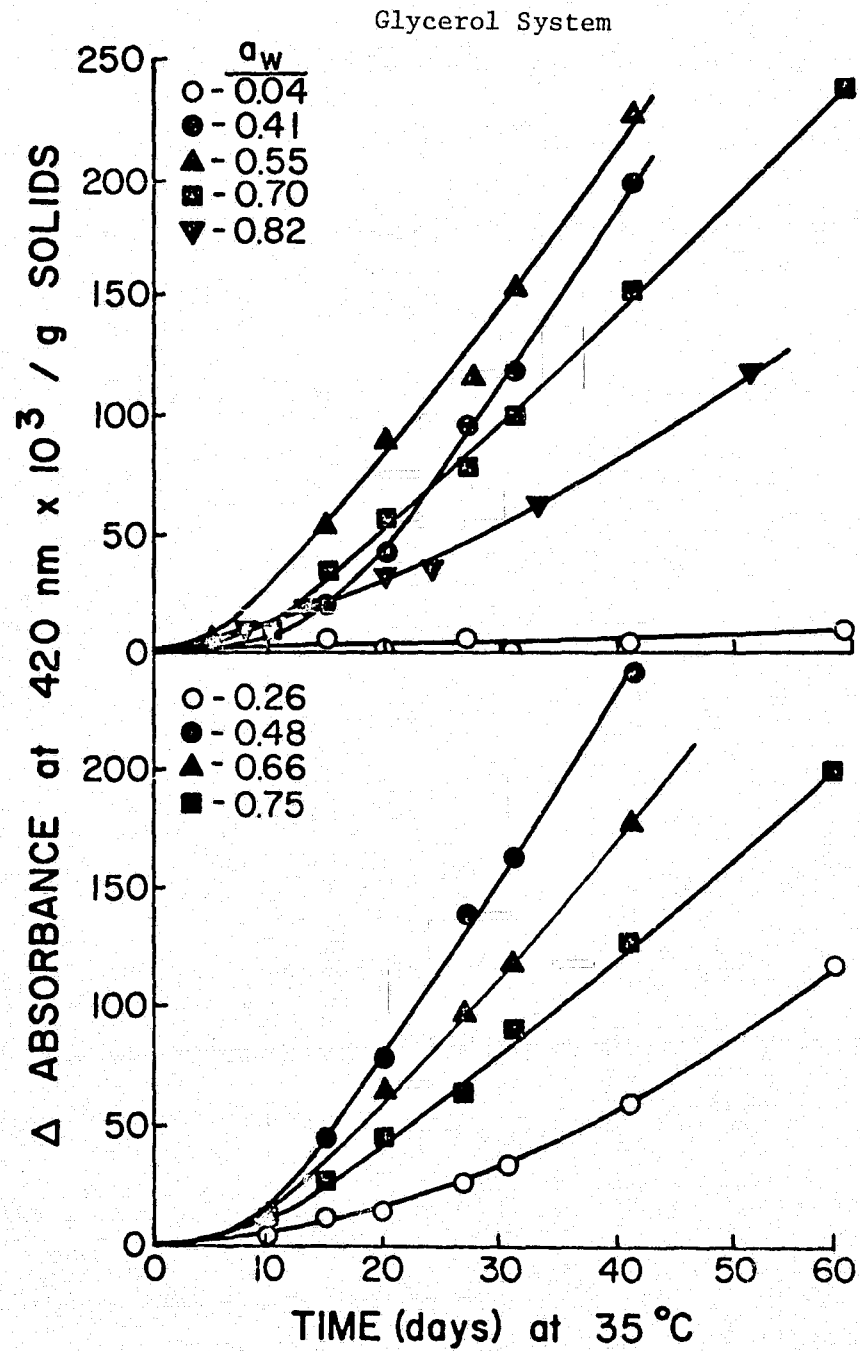
FIGURE 4

RATE OF BROWNING AS A FUNCTION OF a_w AND MOISTURE CONTENT AT 35°C
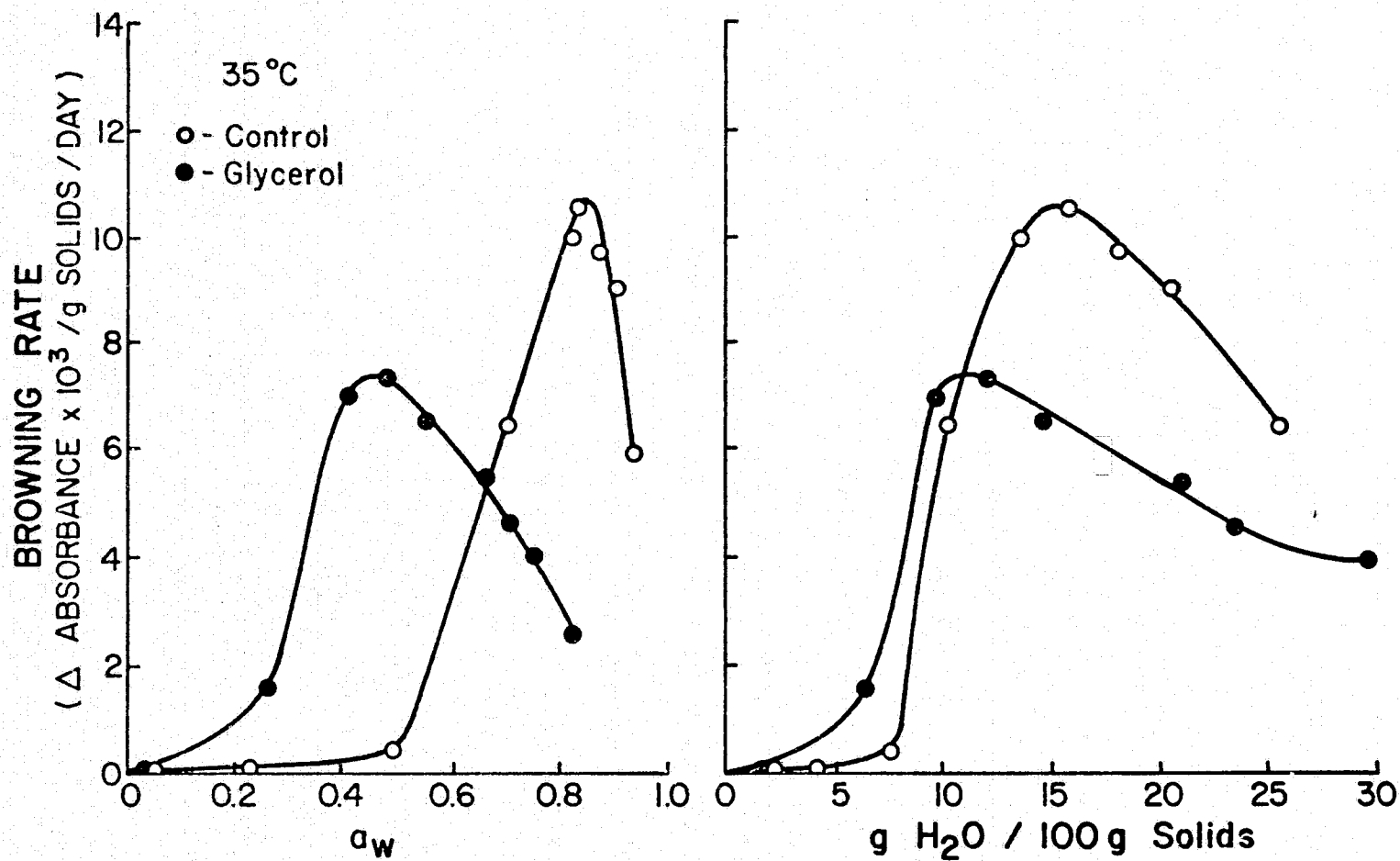
FIGURE 5

RATE OF BROWNING AS A FUNCTION OF a_w AND MOISTURE CONTENT AT 35°C
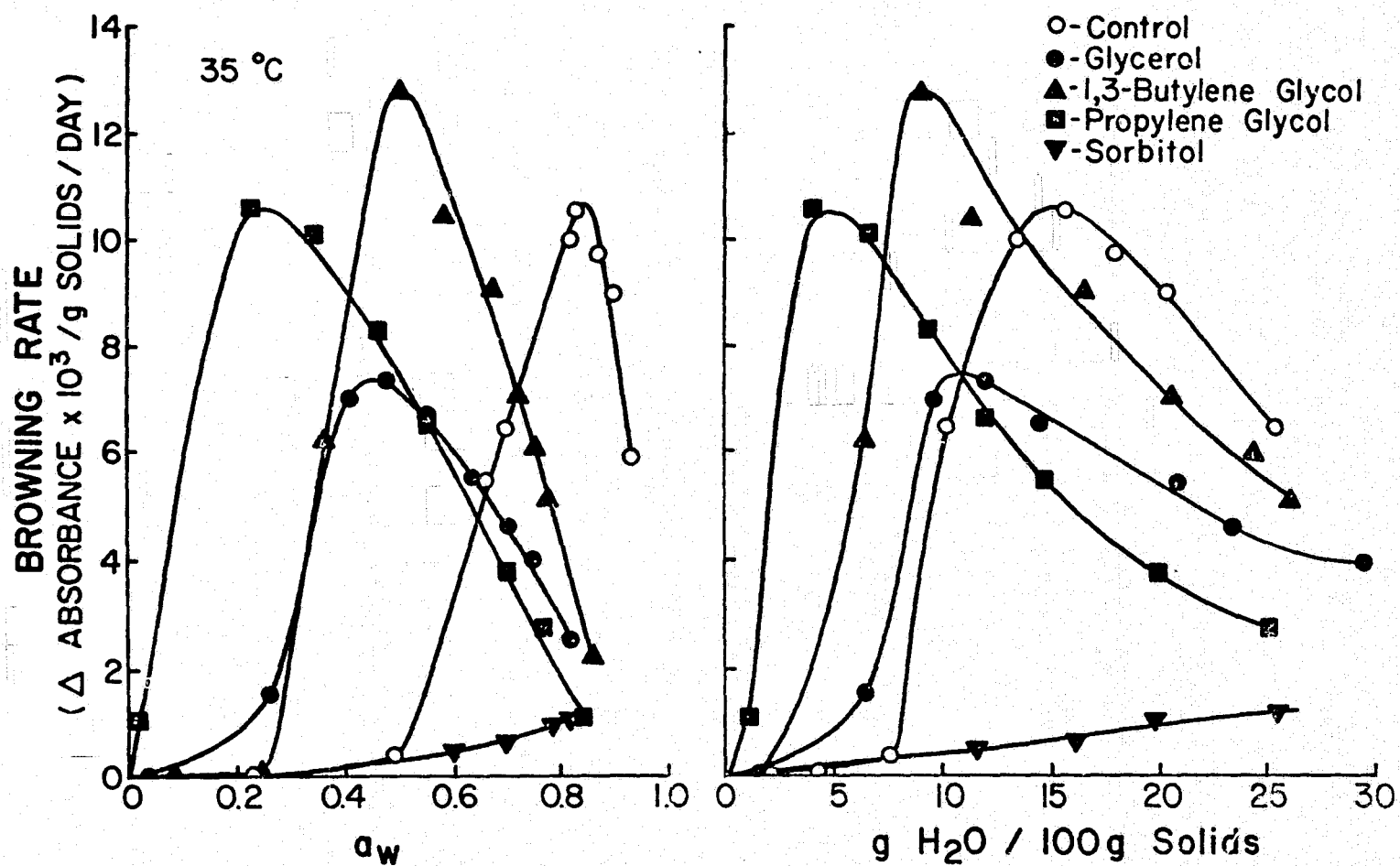
TABLE 3

CONTROL SYSTEM - NEB RESULTS

(Δ Absorbance at 420nm x 10^3/g solids)

a_w	DAYS: 3	9	15	21	30	39	50
0.05	0	8.3	1.5	5.7	3.5	0.5	0
0.23	0	7.9	4.0	6.9	4.0	1.6	1.6
0.49	0	9.8	6.0	7.9	12.5	15.3	24.2
0.70	0	27.7	54.4	92.5	142.4	196.4	298.4
0.82	0	47.1	94.6	167.7	245.1	335.3	456.3
0.83	0	46.1	93.1	148.2	258.1	344.8	473.6
0.87	0	37.1	78.1	137.0	216.6	305.6	435.2
0.90	0	38.8	72.8	122.1	199.6	277.1	387.8
0.93	0	26.9	——	99.5	141.6	197.8	274.7

Glucose Remaining (%)

a_w	DAYS: 3	9	15	21	30	39	50
0.05	89.7	93.4	85.7	97.1	70.7	87.7	87.4
0.23	84.3	87.4	85.1	93.4	71.1	87.8	84.9
0.49	83.2	89.1	85.5	95.3	57.6	89.0	82.9
0.70	83.8	84.2	72.4	77.0	53.5	68.4	65.8
0.82	80.8	73.9	60.5	63.5	42.6	49.1	45.4
0.83	74.3	69.5	58.6	60.8	40.7	47.0	42.2
0.87	77.2	72.1	61.0	65.6	42.8	49.3	44.6
0.90	77.3	75.1	63.7	67.3	46.1	52.8	46.5
0.93	74.2	76.6	65.8	72.1	47.6	58.2	52.1

TABLE 4

NON-ENZYMATIC BROWNING RESULTS: GLYCEROL SYSTEM

(Δ Absorbance at 420nm x 10^3/g solids)

a_w DAYS:	10(8)*	15(13)	20	27(24)	31(33)	41(51)	59
0.04	0	9.3	0.5	18.2	1.5	5.9	12.3
0.26	0	12.2	16.0	31.5	35.7	60.2	123.6
0.41	0	22.9	43.0	96.5	118.1	199.5	345.0
0.48	8.5	47.8	80.0	140.7	165.2	243.8	387.6
0.55	17.0	55.3	81.4	117.2	155.6	228.4	359.7
0.66	13.2	42.6	66.5	98.8	120.2	186.3	361.4
0.70	9.3	35.6	57.9	79.8	101.6	153.9	237.3
0.75	9.8	28.5	47.8	75.2	80.9	127.6	196.0
0.82	8.6	19.7	32.5	35.8	64.4	103.0	———

Glucose Remaining (%)

a_w DAYS:	10	15	20	27	31	41	59
0.04	105.7	110.4	110.2	105.0	102.6	107.3	100.5
0.26	100.9	103.9	102.7	94.8	89.1	89.4	75.4
0.41	98.2	92.2	90.3	79.2	71.6	68.2	52.7
0.48	93.9	86.8	84.0	72.0	63.9	59.8	47.2
0.55	79.9	76.8	75.1	64.6	58.8	54.2	44.4
0.66	81.6	77.5	78.2	66.4	59.4	61.0	48.1
0.70	81.0	83.3	87.4	76.2	71.8	65.8	53.7
0.75	85.8	83.0	88.6	74.3	69.5	· 68.4	59.7
0.82	101.9	100.0	90.6	88.1	80.4	72.6	———

* Days for Abs. at a_w 0.82 in the parentheses

TABLE 5

NON-ENZYMATIC BROWNING RESULTS: BUTYLENE GLYCOL SYSTEM

(Δ Absorbance at 420nm x 10^3/g solids)

a_w DAYS:	3	7	15	21	25	39	50	60
0.09	0	2	0	0	5	0	0	29
0.25	0	3	0	2	15	11	40	65
0.37	0	10	54	98	157	293	450	585
0.51	6	37	162	216	289	416	635	735
0.59	12	42	136	189	255	376	591	712
0.68	9	32	114	159	238	313	580	652
0.73	5	28	96	127	189	266	504	613
0.76	7	23	78	108	166	213	441	500
0.78	4	7	54	79	118	169	367	404
0.86	3	13	32	44	80	96	230	298

Glucose Remaining (%)

a_w DAYS:	3	7	15	21	25	39
0.09	100	101.9	101.1	99.5	98.0	94.8
0.25	100	104.0	104.3	101.7	96.5	82.7
0.37	99.5	98.6	87.9	76.8	70.5	54.0
0.51	96.9	90.2	63.1	56.9	50.8	41.0
0.59	94.2	87.5	68.9	59.0	54.5	44.7
0.68	98.8	94.0	75.7	66.0	58.6	47.5
0.73	95.9	92.6	81.2	72.0	64.4	52.2
0.76	99.3	98.7	80.0	73.3	69.3	54.0
0.78	101.4	98.2	87.8	77.2	72.6	62.9
0.86	99.4	96.4	88.4	81.4	79.6	64.8

-181-

TABLE 6

NON-ENZYMATIC BROWNING RESULTS: PROPYLENE GLYCOL SYSTEM

(Δ Absorbance at 420nm x 10^3/g solids)

a_w DAYS:	5	20	26	40	60	100
0.02	0	17	32	61	155	315
0.24	5	164	230	358	645	969
0.35	11	164	215	352	654	993
0.46	12	137	193	313	613	881
0.55	6	106	164	290	542	867
0.64	6	89	139	258	525	694
0.70	1	59	90	200	375	624
0.77	0	42	66	161	338	497
0.84	0	18	37	114	220	300

Glucose Remaining (%)

a_w DAYS:	5	20	26
0.02	103.8	100.8	102.2
0.24	97.4	65.0	64.9
0.35	85.7	55.2	54.7
0.46	87.8	60.1	59.7
0.55	90.0	62.6	60.5
0.64	86.3	64.1	63.2
0.70	90.7	72.1	70.7
0.77	88.9	75.5	75.6
0.84	96.4	87.0	87.7

TABLE 7

NON-ENZYMATIC BROWNING RESULTS: SORBITOL SYSTEM

(Δ Absorbance at 420nm x 10^3/g solids)

a_w DAYS:	5	20	26	40	60	100
0.05	0	0	12	8	12	11
0.23	0	0	13	5	38	17
0.44	0	0	13	16	42	16
0.55	1	1	12	12	58	23
0.60	0	0	6	15	43	39
0.63	0	0	9	16	51	72
0.71	0	1	9	31	100	173
0.77	1	8	26	67	194	258
0.82	0	10	31	62	183	280

Glucose Remaining (%)

a_w DAYS:	5	20
0.05	98.2	98.6
0.23	105.2	103.0
0.44	103.5	101.5
0.55	101.0	98.5
0.60	100.2	98.4
0.63	94.7	95.0
0.71	90.2	85.0
0.77	96.8	89.7
0.82	88.0	77.8

maximum in browning rate. The maximum, however, has been shifted down towards the monolayer moisture content. The sorbitol sample on the other hand exhibits a very slow rate of browning over the whole a_w range.

The viscosities of the various polyols must contribute partially to the relative differences in the rates of browning when glycols are added. Glycerol, the most viscous, as seen in Table 8, shows the lowest rate at the browning maximum. However, there is no direct correlation with viscosity when compared to the other systems. Glycerol is 10 times more viscous than the butylene glycol but shows only a difference in rate of about 25%. The same situation exists for propylene glycol. Since diffusion rates can be related inversely to the viscosity, one would expect a drop in rate at the higher viscosity but, as seen, no direct function exists.

In addition, the rate of browning is not related directly to total phase volume. If this were true one might expect the rate maximum to occur at the same liquid phase volume in all the systems. This is compared in Table 8 where the phase volume at the rate maximum was calculated based on densities of water-glycol mixtures (i.e. no interaction with the solids). As seen, the phase volumes are about twice as large at the maximum compared to the control system, thus this is not the absolute controlling mechanism. It is interesting, however, that the phase volumes are fairly similar for all the glycols.

Overall, it must be assumed that a number of factors are working together to create the above phenomena.

TABLE 8

PHYSICAL PROPERTIES OF HUMECTANT SYSTEMS

Humectant	Specific volume cc/gram	Viscosity centipoise	Phase volume at rate maximum; cc/100g non-humectant solids
Propylene glycol	0.965	44	31.0
Butylene glycol	0.995	96	36.4
Glycerol	0.793	954	35.0
Sorbitol	0.67	110	——
Control	1.0	1.0	15.6

(1) The glycol, being liquid, must be able to dissolve the reactants to some degree at low moisture contents close to or below the monolayer. Thus, the glycols have properties like water with respect to reaction kinetics. The amount dissolved, however, is not known and must be important from the standpoint of relative rates.

(2) The rate is partially limited by the viscosity of the aqueous phase. The higher the viscosity, the slower the rate. This must partially account for the rate increase as a_w (or H_2O) increases up to the maximum, above that point the water is acting as a diluent. This causes the rate to decrease since the concentration of reactants decreases faster than viscosity decreases.

Sorbitol does not follow this pattern. It is a solid at low a_w. Hence sorbitol acts merely to increase the viscosity of the liquid phase. This reduces the rate of browning at all water activities as seen in Figure 5.

The overall browning rates are summarized in Table 9 for the rate at the maximum and at a_w 0.85, the usual a_w for many intermediate moisture foods. The results indicate that propylene glycol would be the best liquid glycol to add to IMF systems, but would cause serious problems if used as a plasticizer for dry foods. Sorbitol in addition would function as well or better at high a_w but holds less water. The data also indicate that the IM food systems containing glycols should be protected from drying out during storage as this would increase the browning rate.

TABLE 9

COMPARISON OF BROWNING RATES

Humectant	a_w	Browning rate (x 10^3)	
		Per g solids/day	Per g non-glycol solids/day
Control	0.83*	10.6	10.6
	0.85	10.5	10.5
Glycerol	0.48*	7.4	9.5
	0.85	2.1	2.6
1,3 Butylene glycol	0.51*	12.8	16.0
	0.85	2.6	3.3
Propylene glycol	0.24*	10.6	13.3
	0.85	1.0	1.3
Sorbitol	>0.82*	1.2	1.5
	0.85	1.3	1.8

* at maximum

Tables 3 through 7 also contain the data for glucose loss in these systems. The loss pattern follows the same pattern as for color development. Table 10 shows that the maximum for glucose loss occurs close to the maximum for browning rate as would be expected. Based on this and the results from Section D, one can also expect the nutrient loss of lysine to follow the same pattern.

4. References

Choi, R.P., A.F. Koncus, C.M. O'Malley and B.W. Fairbanks. 1949. A proposed method for the determination of color of dry products. J. Dairy Sci. 32: 589.

Eichner, K. and M. Karel. 1972. The influence of water content and water activity on the sugar-amino browning in model systems under various conditions. J. Agric. Food Chem. 20: 218.

Labuza, T.P. 1972. Mechanisms of deterioration of intermediate moisture food systems. NASA Report CR-114861, NASA, Houston, Texas. pg. 18.

Labuza, T.P. 1973. Storage stability and improvement of intermediate moisture foods. Contract NAS 9-12560, Final Report, NASA, Houston, Texas. pg. 57.

Labuza, T.P. 1974. Storage stability and improvement of intermediate moisture foods. Contract NAS 9-12560, Phase II, Final Report, NASA, Houston, Texas. pp. 157-193.

Loncin, M., J. Bimbenet and J. Lenges. 1968. Influence of water activity on the spoilage of foods. J. Food Technol. 3: 131.

TABLE 10

COMPARISON A_W MAXIMA

Humectant	Browning	Glucose loss
Control	0.83	0.83
Glycerol	0.48	0.55
1,3 Butylene glycol	0.51	0.51
Propylene glycol	0.24	0.35
Sorbitol	0.82	0.82

F. Rapid Biological Evaluation of Loss of Available Lysine

1. Introduction

Non-enzymatic browning is a result of a reaction between
the ε-amine group of lysine and reducing sugars or compounds. This
results in Maillard compounds that humans possibly are unable to
digest. In Section D it was shown that by chemical means, lysine
becomes unavailable even before visual browning occurred when measured
chemically. The effect of loss of this lysine in the early stages
of reaction either during heat processing or during food storage on
the overall nutritional value of protein in foods is unknown. The
chemical analyses, such as the Carpenter-Booth fluorodinitro benzene
(FDNB) assay, used to measure the loss of available lysine show a
decrease but it is unknown whether the same would show up in a rat
protein efficiency ratio (PER) test. A bioassay may not show
unavailability in the early stages of the Maillard reaction if the
compound is broken down during digestion. To study this using PER
would be prohibitive thus a microbiological assay is needed.

A microassay using a protozoa, Tetrahymena pyriformis W.,
is such a procedure requiring only four days and minimal sample. This
report reviews work done with Tetrahymena and outlines procedures for
obtaining a reference growth curve for casein in our laboratory.

Tetrahymena pyriformis W is a unicellular, ciliated
protozoa with the same essential amino acid requirements as man, as
shown in Table 1 (Hill, 1972). They are able to digest intact proteins,
however, some workers find better correlation to rat PER values if
an enzymatic digestion with pepsin or papain is utilized (Porter, 1973).

TABLE 1

NUTRIENT REQUIREMENTS FOR <u>TETRAHYMENA</u> <u>PYRIFORMIS</u> W

Essential Amino Acids

Lysine
Arginine
Histidine
Isoleucine
Leucine
Methionine
Phenylalanine
Threonine
Tryptophan
Valine

Essential Vitamins

Thiamine
Riboflavin
Pyridoxal
Pantothenate
Folate
Niacin
Lipoic acid (thioctic acid)

Essential Inorganic Nutrients

Phosphate
Magnesium
Potassium
Copper

Essential Nucleic Acids

Guanine
Uracil or Cytidine or Cytidylic acid

Although native to pond water, the organism can be grown on a chemically defined media and protein source.

Early workers classified the organism as _Glaucoma ficaria_ and _Tetrahymena geleii_ until it was more correctly classified as _T. pyriformis_ W. There are over 20 strains of _T. pyriformis_ that are unicellular ciliates of the subclass Holotricha, but they differ in mean body size, thermal resistance and amino acid requirements.

The essential nutritional requirements are found in Table 1 (Hill, 1972; Kidder, 1940). Growth is inhibited by free fatty acids, so these need to be extracted from the food samples with ether (Fernell, 1956) or chloroform and methanol. Carbohydrates are not essential, but provide optimal growing conditions and restrict the use of amino acids for energy. The ratio of phosphates to vitamins is important both to encourage growth and to prevent excessive growth (Fernell, 1956). Some particulate material is required for rapid growth (Hill, 1972).

T. pyriformis W produces very little acid during growth, but pH should be buffered between pH 5 and 8.6 for optimum growth. Temperature should be maintained at $25^{\circ}C$. Growth is normally found in the temperature range of 18 to $28^{\circ}C$. Reproduction occurs by binary fission every 10-20 hr, therefore, most tests are run for 4 days (96 hr) which gives about a two log cycle increase in population.

2. Materials and Methods

The method followed is primarily that of Stott and Smith (1966) and Landers and Ashton (1975).

a.   Test organism

Tetrahymena pyriformis W (10542) is available from
the American Type Culture Collection (12301 Parklawn Drive, Rockville,
Maryland 20852). One ml of a stock culture is transferred weekly into
10 ml of nutritional broth in a 15 x 190 test tube. This broth
consists of 2% protease peptone, 0.1% yeast extract, 0.5% glucose,
and 0.1% sodium chloride adjusted to pH 7.1 with sodium hydroxide
and autoclaved 15 minutes at 121°C. Normal growth of the organism is
shown in Figure 1. Inoculation of samples are made with 0.1 ml of a
three or four day broth delivered from a one ml blow-out pipette
graduated in .01 ml.

b. Media preparation

Stock solutions of vitamins (Solution A) and minerals
(Solutions B, C, D), citric acid and buffers are prepared and stored
as indicated below. Media for each run is prepared the day of use,
or in some cases, on the previous day and refrigerated. Figure 2
provides a flow chart for preparation and autoclaving for 10 min at
121°C.

(1) Vitamins

The vitamins in the amounts shown in Table 2
are weighed and dissolved in distilled water. Riboflavin, folic acid,
and p-amino benzoic acids in particular need to be dissolved in water
heated to 55°C with stirring. The solution is then frozen in 7 ml
portions in 2 oz. jars. The frozen vitamin solution is stable for
several months, whereas fresh preparations may need to be observed
for deterioration or mishandling of the reagents.

FIGURE 1

NORMAL GROWTH PATTERN OF TETRAHYMENA PYRIFORMIS W
AT 25°C IN STOCK SOLUTION
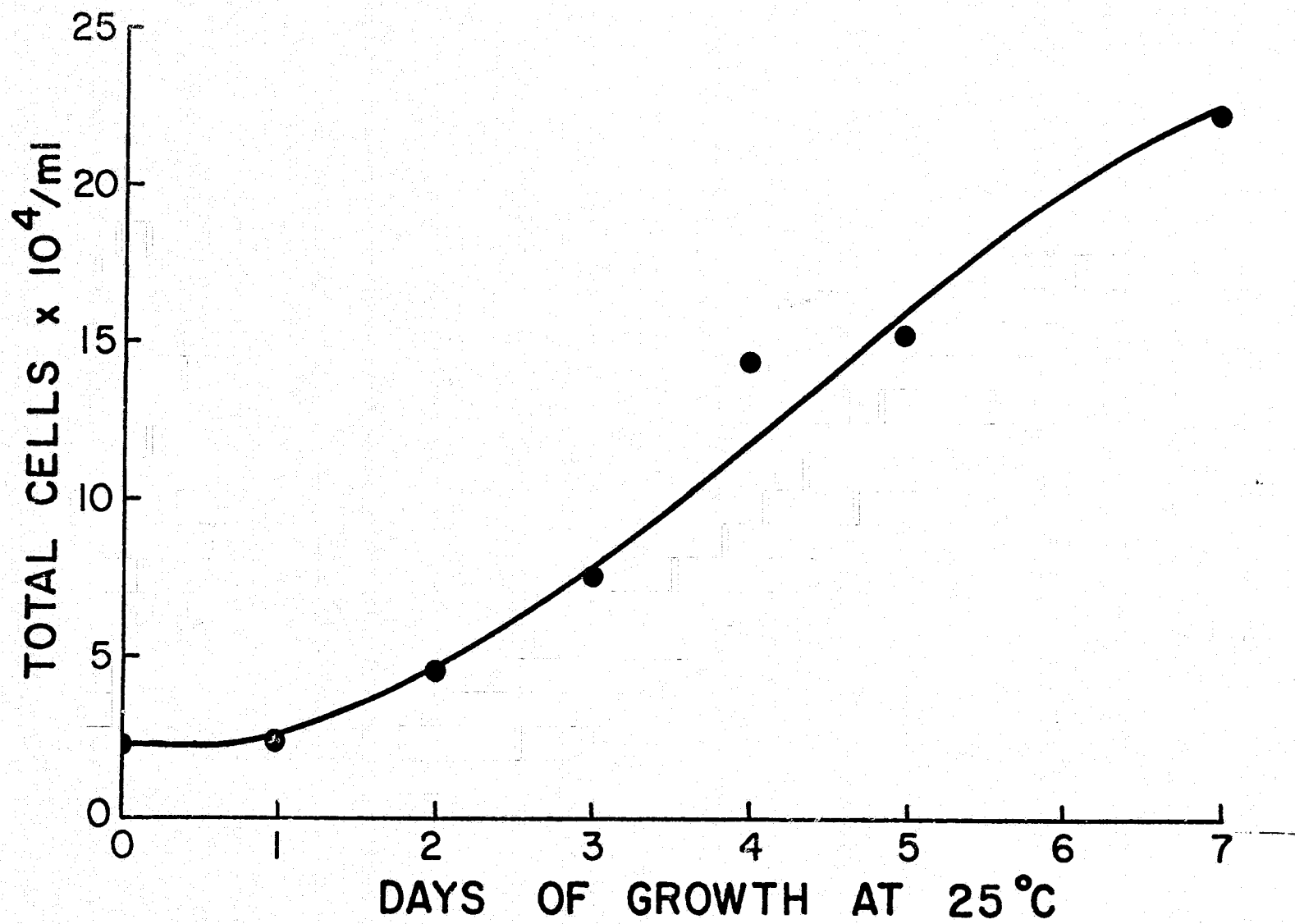
FIGURE 2

FLOW CHART OF <u>TETRAHYMENA</u> ASSAY

nucleic acids
water
citric acid
solutions B,C,D

2 ml →

3 ml ← digested casein
water

autoclave

autoclave ← vitamins

5 ml ←

autoclave ← glucose

0.1 ml TPW

4 days - 25°C

1 ml →

preserving
fluid

1 ml

1 ml → microscopic
count
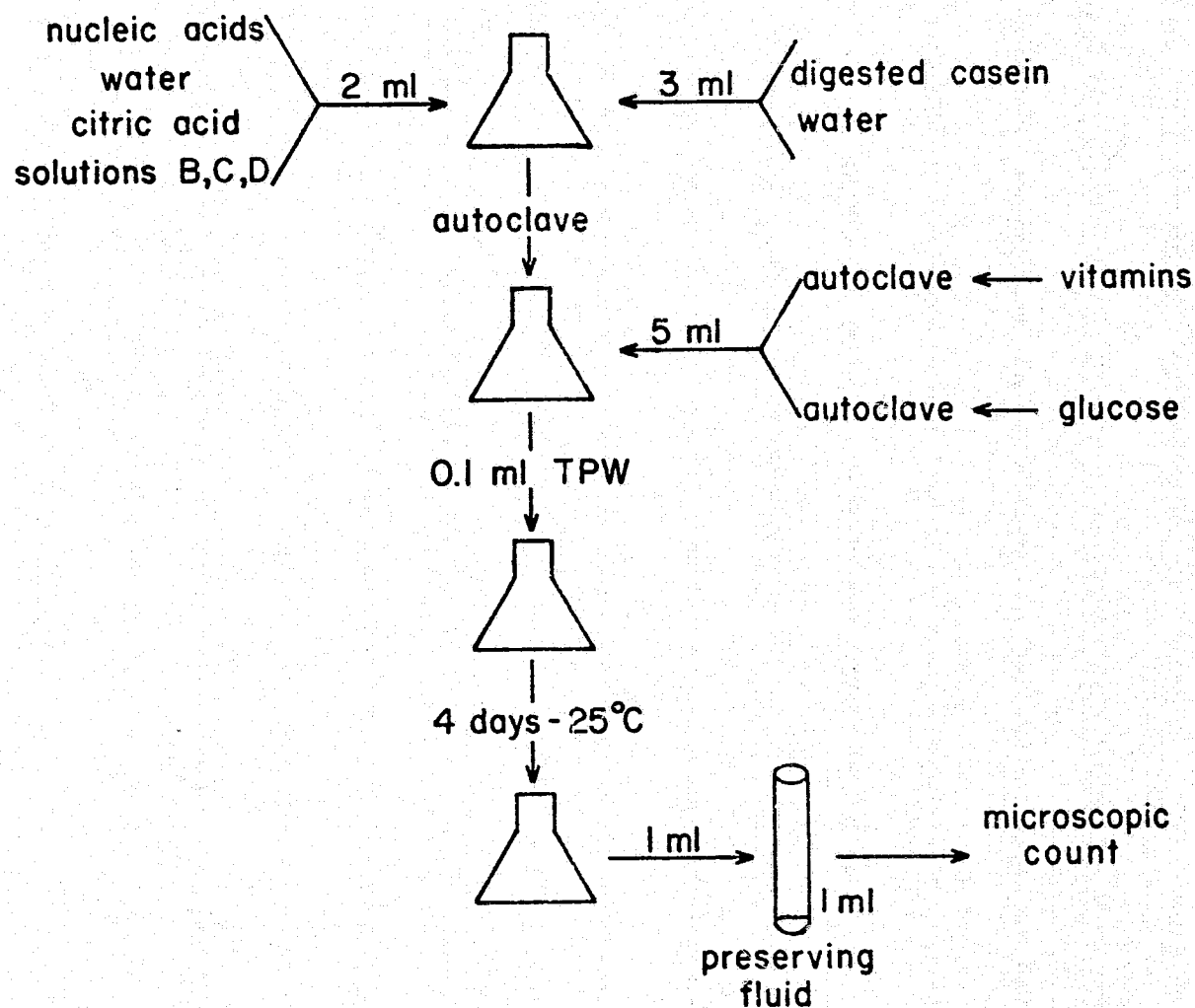
TABLE 2

NUTRIENT SOLUTIONS

Vitamin Solution A

Vitamin	mg/200 ml
Calcium pantothenate	12.5
Nicotinamide	12.5
Pyridoxine hydrochloride	125.0
Pyridoxal hydrochloride	12.5
Pyridoxamine hydrochloride	14.8
Riboflavin	12.5
Folic acid	1.25
Thiamine hydrochloride	125.0
Inositol	12.0
Choline chloride	125.0
p-Amino benzoic acid	12.5
DL- Lipoic acid (thioctic acid)	0.4

Nucleotide Solution E

	mg/20 ml
Guanylic acid (sodium salt)	15
Adenosine – 2'(3') – phosphoric acid monohydrate	10
Cytidylic acid	12.5
Uracil	5

On the day of sample analysis, the frozen vitamin stock A is thawed at room temperature and 2.4 ml are made up to 25 ml with distilled water. This solution is then autoclaved. Simultaneously a glucose solution of 3.6 g of glucose (dextrose) and 95 ml of distilled water is dissolved in hot water and autoclaved. After autoclaving, these two solutions are combined and 5 ml delivered aseptically to each sterile sample flask with a syringe, as indicated in Figure 2.

(2) Nucleic acids - mineral solution E

This solution is prepared on the day of analysis by weighing the quantities indicated in Table 2 and combining with stock solutions B, C, D, citric acid and buffers according to Table 3. Two ml of this mineral-nucleotide solution is then delivered to each sample flask with a 3 ml disposable syringe.

Personal communication with Landers and Ashton (Campbell Institute for Food Research) indicated they prepared this solution slightly differently than the procedure used by Stott and Smith (1966). Primarily, Landers and Ashton have added citric acid and Tris buffer and have 10 times the quantity of Solution D and a final concentration 25% more dilute than used by Stott and Smith. Figure 3 represents the difference obtained in our lab using the two concentrations of nucleic acids and phosphates. System A represents the procedure and concentrations of Stott and Smith. System B represents the procedure of Landers and Ashton equivalent to 9.96 (10 ml) of Solution D to 20 ml of mineral nucleotide solution, resulting in lower growth of Tetrahymena. This difference can be attributed to lower concentrations of nucleic acids and an increased ratio of phosphates

TABLE 3

MINERAL AND BUFFER SOLUTIONS

Stock Solution B*

	g/200 ml
$MgSO_4 \cdot 7H_2O$	2.8
$Fe(NH_4)_2(SO_4)_2 \cdot H_2O$	1.25
$MnCl_2 \cdot 4H_2O$	.025
$ZnCl_2$	.0025

Stock Solution C*

	mg/200 ml
$CaCl_2 \cdot 2H_2O$	600
$CuCl_3 \cdot 6H_2O$	60
$FeCl_3 \cdot 6H_2O$	15

---

* Store in refrigerator

Stock Buffer Solution D*

100 ml Solution D_1 (0.2 M KH_2PO_4 + 0.2 M K_2HPO_4 to give pH 7.1)

100 ml Solution D_2 (0.2 M pH 7.1 Tris HCl Buffer (hydroxy-
methyl amino methane)

0.02 M citric acid solution (store in refrigerator)

Dissolve nucleotides (Table 2) in 10 ml of hot distilled water.
Add 1 ml each of stock solution B, C, D and .02 M citric acid.
Adjust volume to 20 ml and adjust pH to 7.1 with 1.0 N NaOH.

FIGURE 3

DIFFERENCE IN TETRAHYMENA GROWTH DUE TO
CONCENTRATIONS OF NUCLEIC ACIDS IN MEDIA
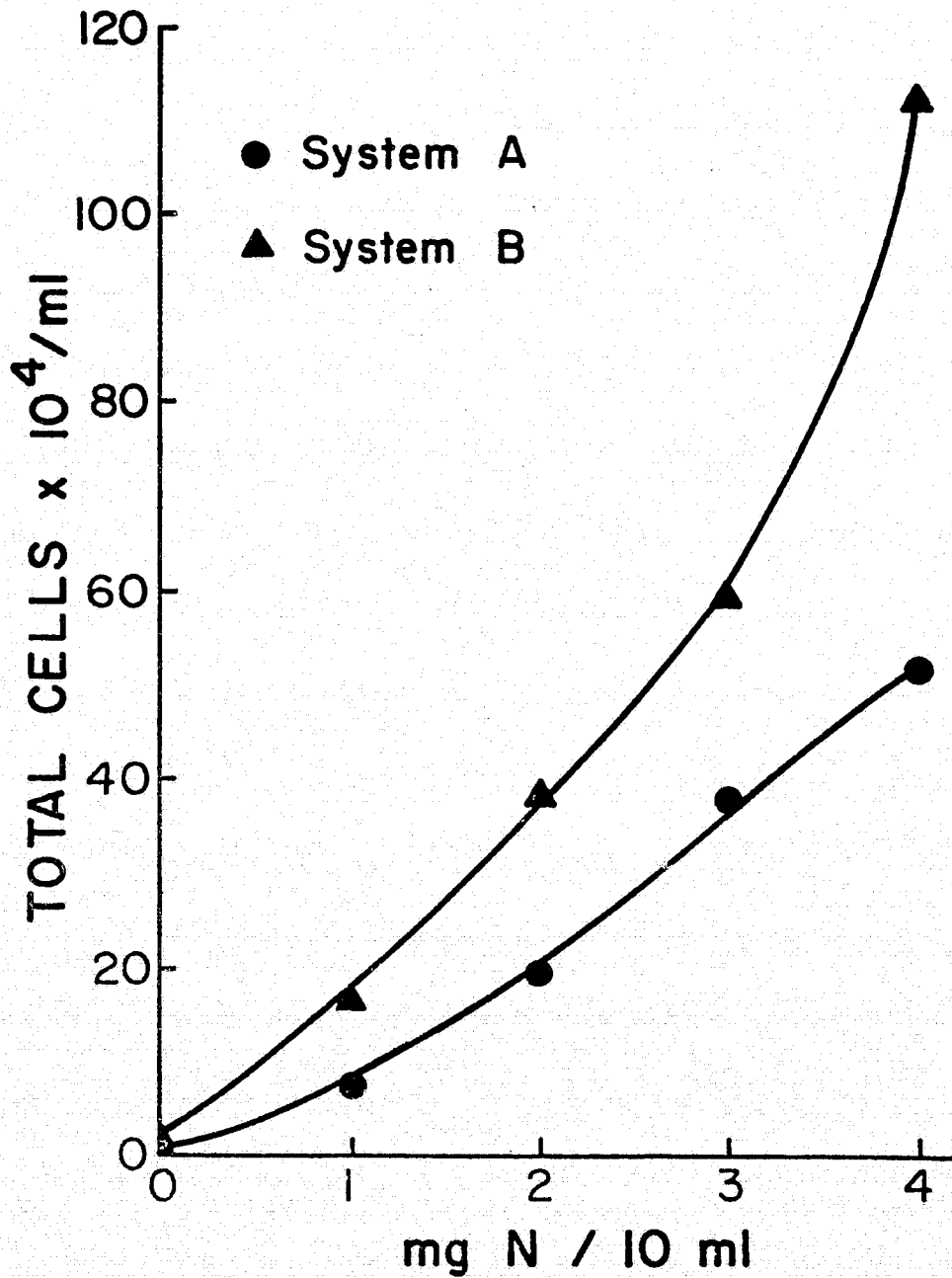
to vitamins, variables also noted by Fernell (1956).

(3) Casein (reference sample)

Sample preparation normally would require extraction of lipid material with 3:1 (v/v) chloroform/methanol, drying under mild conditions (24 hr freeze drier), and pepsin digestion. Pepsin digestion consists of using 1 ml of 1% pepsin, a sample weight equivalent to 100 mg nitrogen and 0.05 N HCl for 3 hr at 55°C. The sample is cooled, pH adjusted to 7.1 and the final volume made to 50 ml graduated pipette into 50 ml Erlenmeyer flasks containing the nucleic acid-mineral solution. Samples are then autoclaved 10 min at 121°C.

Originally, an unautoclaved digest was added to the autoclaved nucleotide solution to prevent heat damage. However, a comparison of a casein digest solution, part of which was autoclaved and remainder not sterilized, gave the results shown in Figure 4.

Although lipid extraction and predigestion are not recognized as necessary for casein, these procedures were followed for consistency. Some problems developed in that the casein would not always dissolve during digestion. More careful adjustment of pH to 1.8 before enzymatic digestion seemed to eliminate this problem. Figure 5 shows two samples of caseins analyzed with no apparent protein damage by solvent extraction, or freeze drying.

Media preparation for each experimental run is time consuming. It is preferred that media could be prepared by auto-claving proper amounts of stock solutions in each 50 ml Erlenmeyer with storage overnight or longer at refrigeration temperatures without destroying the essential nutrients. Figure 6 shows three experimental runs of Tetrahymena pyriformis W with casein. Media was prepared on

FIGURE 4

EFFECT OF AUTOCLAVING CASEIN ON GROWTH OF TETRAHYMENA
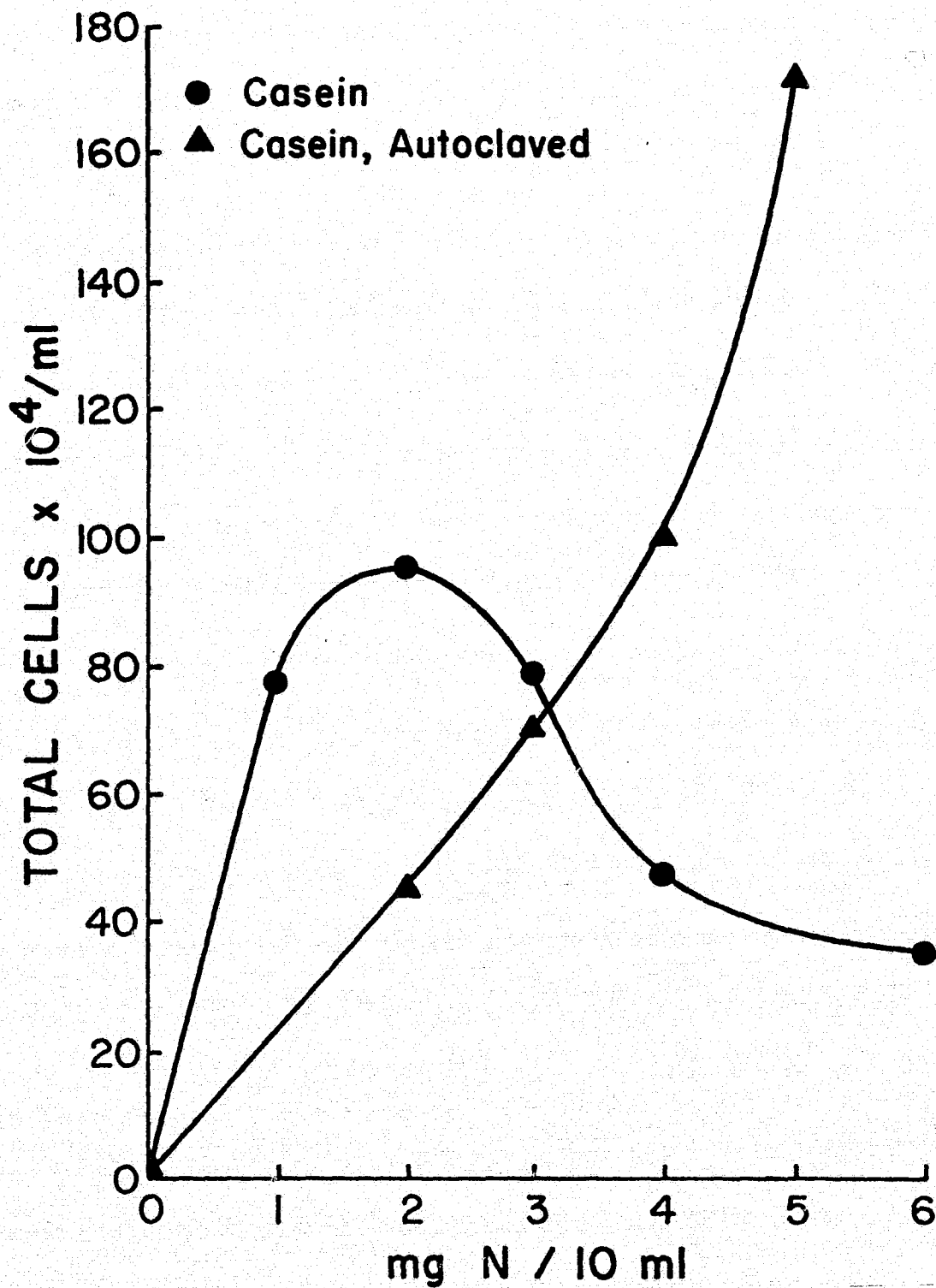
FIGURE 5

EFFECT OF TREATED CASEIN (SOLVENT WASH AND FREEZE DRYING) ON
GROWTH OF TPW AT SYSTEM A NUCLEIC ACID CONCENTRATIONS
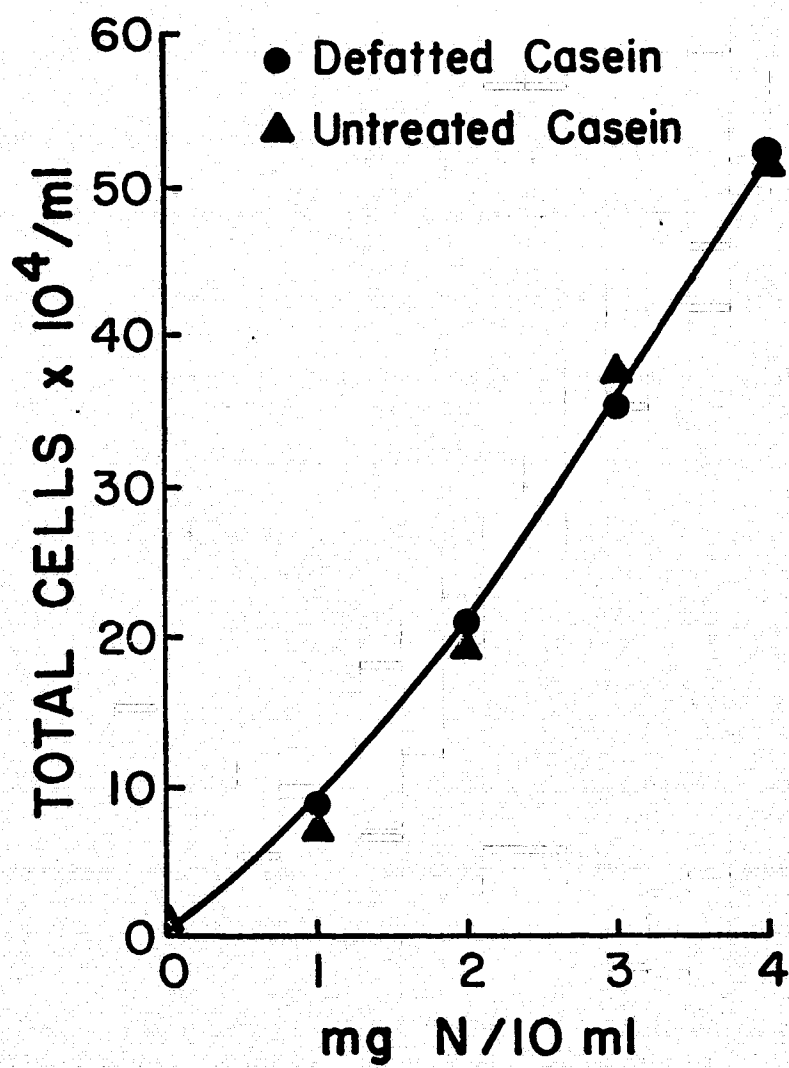
FIGURE 6

EFFECT OF HOLDING PREPARED MEDIA AT 4°C ON <u>TETRAHYMENA</u> GROWTH

(DAY SYMBOLS INDICATE HOLDING TIME)
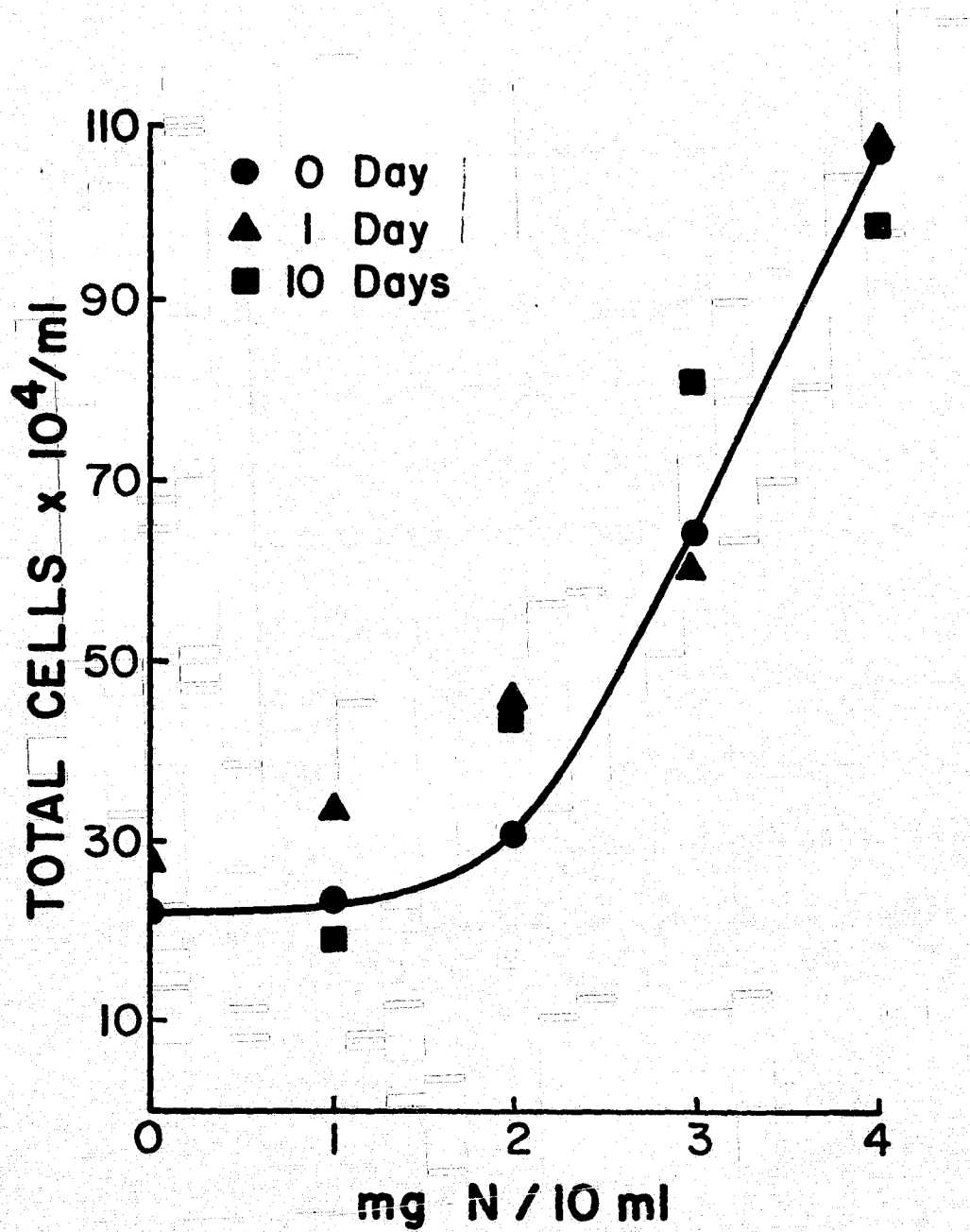
Day 1 by steaming all solutions (except sample casein) at 100°C for 10 min. The temperature of 100°C rather than 121°C of autoclaving was chosen to see in the heat would destroy contamination without heat damage to the nutrients. One run was started on Day 1, another 24 hr later, and the third used the remaining media 10 days later. Although a slight variance in grwoth is observed, the refrigerated media support equal, if not greater, growth. This variance could be due to initial inoculant count or other factors. Studies on precision between duplicate runs have not been run as yet, but it can be seen that at between 2-4 mg N/10 ml greatest sensitivity exists.

(4) Environmental conditions

It should be noted that this batch of media, used on Day 1 and Day 2 (Figure 5), was the only media that prompted the organism to grow in patterns. Although the single layer of cells on the surface film appeared to be a contaminant, they were, in fact, a typical growth pattern for Tetrahymena in shallow solutions (Hill, 1972). The cells will form a distinct layer until they are ready for cell division. During log growth, both old and young cells will form patterns if undisturbed. In the stationary phase, the very old cell will be found near the bottom. As this appears to be a result of using a surface area for needed oxygen, and clumping created problems in pipetting and counting, methods were undertaken to provide shaking in a shaking water bath.

Three vessels have been used for this Tetrahymena assay. Most successful has been 50 ml Erlenmeyer flasks with sterile gauze stoppers. These fit into the shaking apparatus for studies on the affect of aeration and can provide fairly good growth without shaking. An attempt to use 2 oz medically flat screw-cap jars

provided good correlation to growth in Erlenmeyers - but the assay material dried up and concentrated via evaporation. Unsuccessful results were also obtained using test tubes with plastic stoppers as seen in Figure 7. Improved growth was observed in Erlenmeyers by microscopic evaluation of individual cells, as well as total cell count. Tetrahymena grown in test tubes were small and slender. Those grown in the Erlenmeyer flasks were fat and round with large food vacuoles, and often observed in the state of binary fission. The growth curve obtained from Erlenmeyer counts is similar to that obtained by Rosen and Fernell (1956) in their early studies. Growth curves from test tube cells show early, limited growth.

e. Growth measurement

Total cell count is used to determine growth response of Tetrahymena pyriformis W. Microscopic examination (43 x 10 power, light microscope) of the cells in a double cell 0.2 mm deep haemocytometer with Fuscho-Rosenthal gradients is used to count them. The grid is divided into 4 x 4, one square millimeter gradients, with each millimeter sub-divided into 16 squares. The count is calculated as follows:

$$\text{average count per mm}^2 \times \frac{1}{0.2 \text{ mm depth}} \times 2 \text{ dilution factor}$$
$$\times \frac{10^3 \text{mm}^3}{\text{ml}} \times 10 = \text{organisms} \times 10^4 \text{ ml}$$

Turbimetric measurements would be more rapid than cell counts, however, results were unsuccessful. Samples were removed on Day 4 of the experiment for microscopic cell counts, were steamed, cooled and read on a Coleman Jr. spectrophotometer at 580 mµ against water. Considerable drifting occurred, and blank and sample readings

FIGURE 7

GROWTH OF TETRAHYMENA IN TEST TUBES VS.
50 ml ERLENMEYER FLASKS
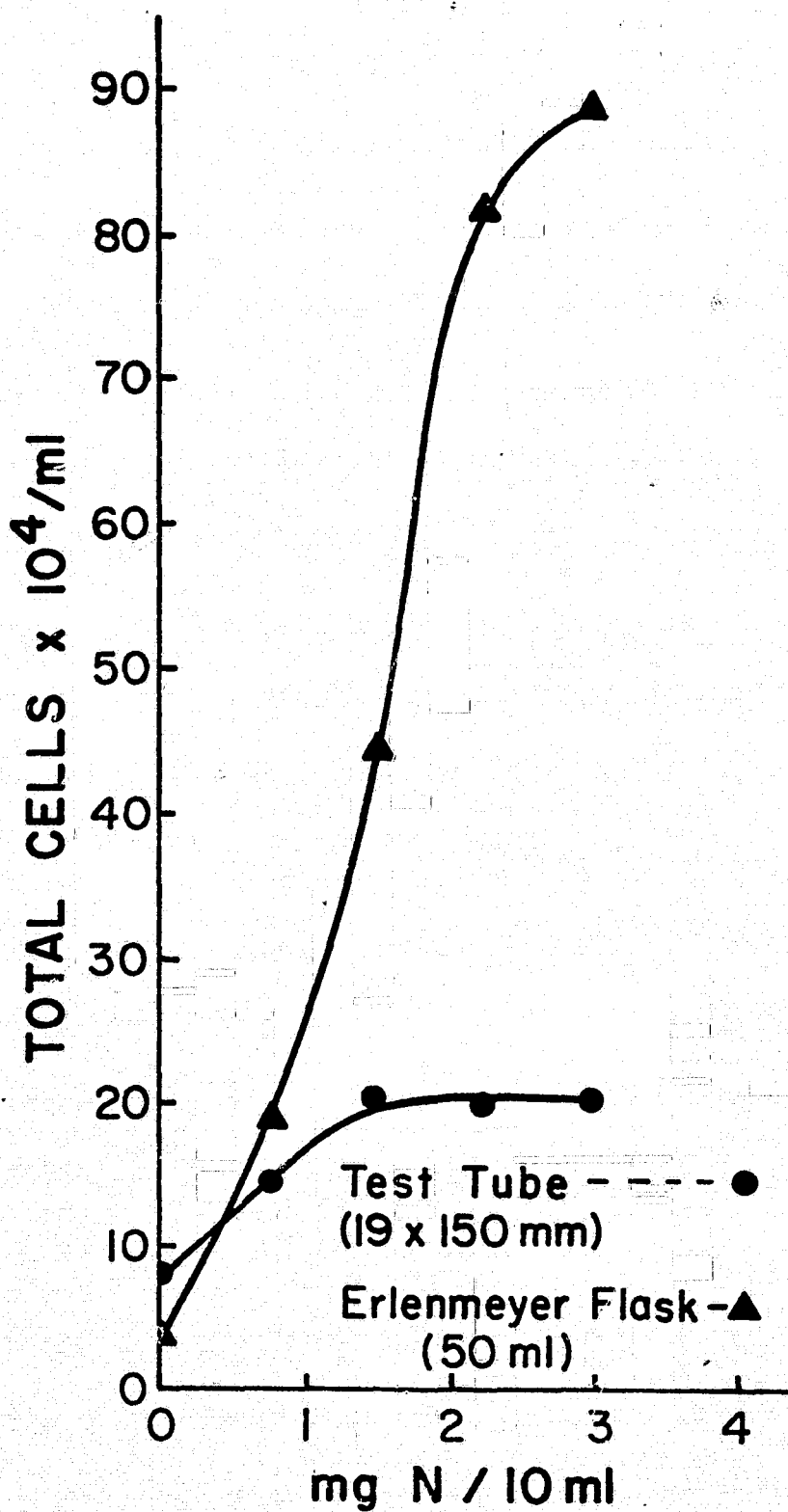
were high, since lower nitrogen levels at 0, 0.1, 0.2, 0.3 and 0.4 mg/10 ml should be used. Drifting was minimized by vortexing the steamed sample for 30 sec to disperse cell contents and reading spectrophotometrically one min later. A complete run of values were read over a period of $2\frac{1}{2}$ min and indicated that one min was the earliest stable reading.

Even with minimal drift, readings tend to be inconsistent using triplicates as seen in Figure 8. Cell count is thus the preferred method of measuring growth for these studies. Microscopic examinations are useful for both high and low ranges of results, and also allow observation of the relative cell size and health of Tetrahymena. Aliquots of samples to be counted can be kept in preserving fluid until time is available or can be saved to check values at a later date.

3. Results and Discussion

The many possible variables in this Tetrahymena pyriformis W. assay have been investigated before any actual experiments on protein loss have been undertaken. It is extremely important that changes in media preparation and growth conditions be kept to a minimum to avoid introducing error. It has been observed that new preparations of vitamin solutions, for example, may affect the total maximum growth. It would be advisable to prepare sufficient stock reagents for a complete experiment to be run during a one to two month period, and to run actual controls of an old stock reagent each time a new is prepared.

The assay procedure as outlined in the previous section should be followed exactly. Autoclaved nucleic acids, casein, vitamins

FIGURE 8

VARIATION IN MEASUREMENT OF <u>TETRAHYMENA</u> BY OPTICAL DENSITY
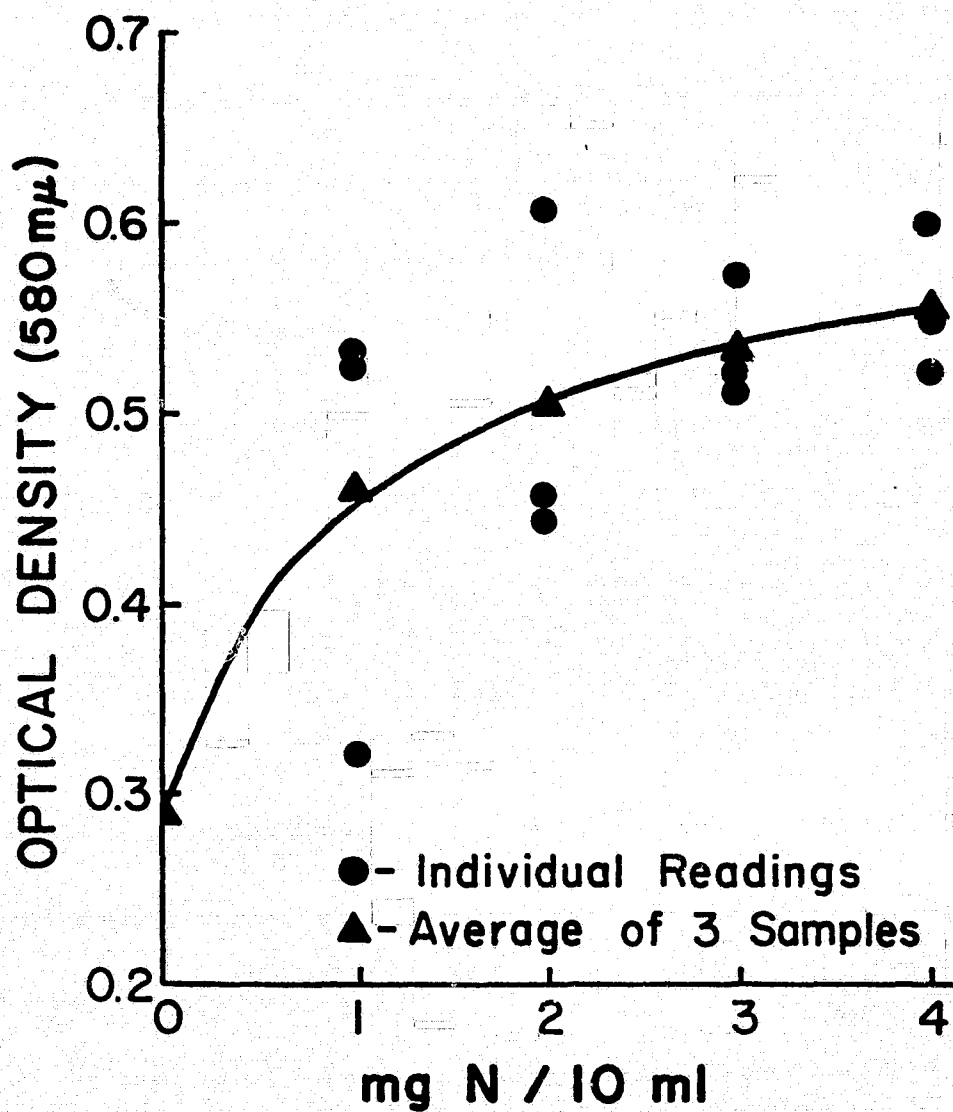
and glucose can be refrigerated separately and combined within a 24 hr period quite satisfactorily, but a control or reference should always be included in each experiment for basis of comparison. Four lysine standard curves were attempted on three different days. Unfortunately, growth was barely more than that of the inoculum. The procedure for lysine standard curve is very similar to a sample casein growth curve. With the same concentration of vitamins and glucose, a separately autoclaved amino acid solution containing no lysine as seen in Table 4 is added. Increments of L-lysine hydrochloride are added instead of casein, and a curve similar to that obtained by Stott and Smith (1966) in Figure 9 should be observed. As this has not been the case, it is possible that an amino acid imbalance has been created, or that the organisms grow preferentially on media with intact proteins.

    4. Conclusions

Environmental conditions, media preparation and the casein reference are prime sources of possible error. Bacteria and mold contamination may inhibit _Tetrahymena_ growth or mask the field of count. Clumping due to stationary cells without aeration prevents accurate subsampling for cell counts. Temperatures should remain constant at 25°C, as optimum growth will decline at other temperatures. To maintain these proper environmental conditions, a shaking incubator is needed at constant temperature.

It is important to prepare media solutions carefully in this assay. Small changes in pH, ionic strength, or concentrations may limit growth of the organisms. New stock solutions of vitamins should

TABLE 4

AMINO ACID SOLUTION

DL-Alanine	L-Proline
L-Arginine hydrochloride	L-Leucine
L-Aspartic acid	L-Cystine
Glycine	DL-Serine
L-Glutamic acid	L-Threonine
L-Histidine hydrochloride	L-Tryptophan
L-Isoleucine	DL-Valine
L-Methionine	L-Lysine hydrochloride

FIGURE 9

LYSINE STANDARD DURVE FOR TETRAHYMENA AS OBTAINED
BY STOTT AND SMITH (1966)
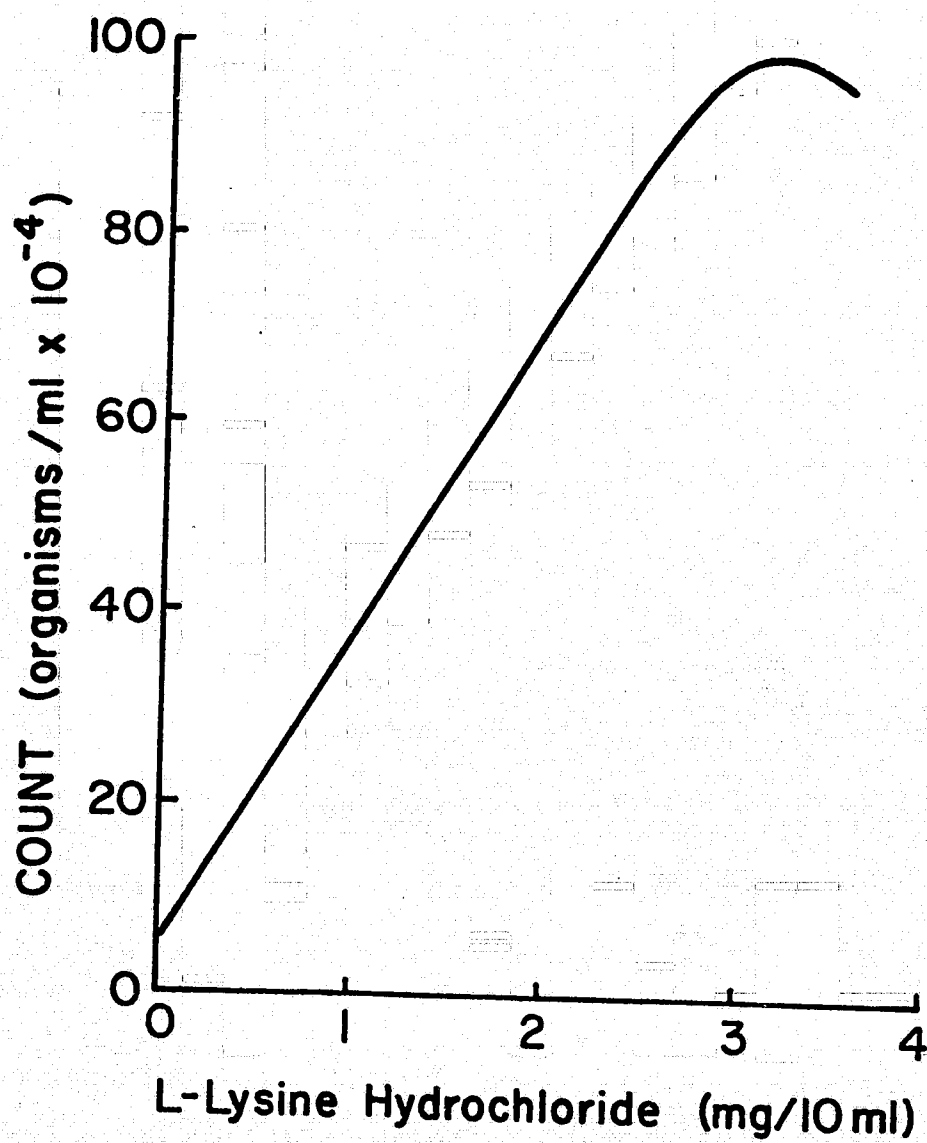
be prepared carefully, sterilized and stored frozen to prevent their degradation. If heat sterilization is damaging, cold sterilization (millipore filtration) can be used. ANRC Reference Casein will be obtained and used in future experiments.

Also, by close monitoring of amino acid solutions, imbalance may be avoided and a lysine standard determined. However, we may find that using relative nutritional values (RNV) of a model food system compared to casein may be sufficient to show nutritional effects of non-enzymatic browning on protein quality. To compare a standard curve based on free amino acids to casein (see Figure 9), we would also need to consider a similar standard curve based on a solution of amino acids corresponding to the composition of casein. Values obtained for casein on curves in Figure 9 are 8.1g available lysine/16g nitrogen (Sherrock and Ford, 1973) and 8.7g available lysine/16g nitrogen (Stott and Smith, 1966). This indicates samples assayed in the range of 0.5 mg lysine/mg nitrogen. Sinclair and Hollingsworth (1969) list the composition of casein as 497 mg lysine/ g nitrogen (equivalent to 7.95g lysine/16g nitrogen). Considerable experimentation will be needed to apply results of available lysine standard curves, whereas casein-glucose non-enzymatic browning lysine loss could be compared directly to the undamaged casein reference. This appears to be a more direct approach to study lysine loss.

To determine if heat-damaged lysine bound to reducing sugar is utilized by Tetrahymena, a dry casein-glucose sample will be autoclaved for 30 min. Growth response to the high heat damaged is expected to be considerably lower than the untreated samples. If

this is observed, samples of intermediate moisture foods using casein

and glucose will be prepared and stored at low temperatures and

assayed for effects of lysine binding.

5. References

Anderson, M.E. and H.H. Williams.   1951.   Microbiological evaluation
      of protein quality.   J. Nutr. 44: 335.

Barton-Wright, E.C.   1952.   The Microbiological Assay of the Vitamin
      B-Complex and Amino Acids.   Pitman Publishing Corp., New York.
      pp. 91-122.

Bender, A.E., R. Kihlberg, B. Lofqvist and L. Munck.   1970.   Evaluation
      of Novel Protein Products.   Pergamon Press, New York.   pg. 235.

Boyne, A.W., S.A. Price, G.D. Rosen and J.A. Stott.   1967.   Protein
      quality of feeding stuffs.   Brit. J. Nutr. 21: 181.

Carliss, J.O.   1952.   Comparative studies on Holotrichous Ciliates in
      the Colpidium - Glaucome - Leucophyrs - Tetrahymena group.   AM.
      Micro. Soc.   71: 159.

Carpenter, K.J. and V.H. Booth.   1973.   Damage to lysine in food
      processing: Its measurement and its significance. Nutr. Abs.
      & Rev.   43: 424.

Fernell, W.R. and G.D. Rosen.   1956.   Microbiological evaluation of
      protein quality with Tetrahymena pyriformis W.   Brit. J. Nutr.
      10. 143.

Ford, J.E.   1962.   The measurement of "available" methionine, leucine,
      isoleucine, arginine, histidine, tryptophan and valine.   Brit.
      J. Nutr.   16: 409.

Hill, D.L.   1972.   The Biochemistry and Physiology of Tetrahymena.
      Academic Press, New York.

Itoh, Keisuke Kowashima and Ichito Chibata.   1973.   Rapid microbiological
      assay of amino acids.   Agric. Biol. Chem.   37: 2227.

Kidder, G.W., D.M. Lilly and C.L. Claff.   1940.   Growth studies on
      ciliates.   Biol. Bull. 78: 9.

Nutritional Meetings Report Series No. 52.   1973.   Energy and protein
      requirements.   FAO.   Rome

Pilcher, H.L. and H.H. Williams.   1954.   Microbiological evaluation of
      protein quality.   J. Nutr.   53: 589.

Porter, J.W.G. and B.A. Rolls. 1973. <u>Proteins in Human Nutrition</u>.
     Academic Press, New York.

Rockland, I.E. and M.S. Dunn. 1949. Determination of the biological
     value of proteins with <u>Tetrahymena geleii</u>. W. Food Technol. 3: 289.

Rogers, C.G., J.M. McLaughlin and D.G. Chapman. 1959. Evaluation of
     proteins in foods. Can. J. Biochem & Phys. 37: 1351.

Sinclair, H.M. and D.F. Hollingsworth. 1969. <u>Hutchinson's Food and
     the Principles of Nutrition</u>. Edward Arnold Publishers, London.

Stott, J.A., H. Smith and G.D. Rosen. 1963. Microbiological evaluation
     of protein quality with <u>Tetrahymena pyriformis</u> W. Brit. J.
     Nutr. 17: 227.

Stott, J.A. and H. Smith. 1966. Microbiological assay of protein
     quality with Tetrahymena pyriformis W. Brit. J. Nutr. 20: 664.

Landers, R.E. and D.H. Ashton. 1975. Personal communication.
     Campbell Institute for Food Research. Campbell Place, Camden
     New Jersey 08101.

G. Summary and Recommendations

Non-enzymatic browning occurs very rapidly in intermediate moisture food systems. This study was designed to look at various aspects of browning. The recommendations are as follows:

1. Protein substitution in IMF may create problems from a nutritional loss and color development standpoint. Thus, one should do a rapid shelf life study in the newly reformulated food.

2. Casein and fish protein concentrate can be freely substituted if they give similar functionality. The same is true for wheat gluten with egg albumin.

3. Liquid glycols such as glycerol, 1,3-butylene glycol and propylene glycol function as browning inhibitors in IMF at 20% of the solids. Sorbitol has a similar effect. Studies at lower concentrations are necessary however.

4. The method of addition of water to a system does not affect browning rates.

5. Browning has a high Q_{10} of 5 to 6 thus high temperatures during processing and storage should be avoided.

6. Lysine degradation occurs rapidly in IMF with 20-60% loss before color changes occur. Thus one cannot base nutritional loss on NEB pigment production.

7. Tetrahymena pyriformis W may be a useful organism for a rapid evaluation of protein nutritional losses.

V. Measurement and Control of Water Activity

A. Introduction

Two major problems exist in manufacture of IMF; choice of a humectant and measurement of the a_w of the finished product. This section contains the papers summarizing the results of studies of humectants and a_w measurement. Work presented under Phase II showed a need for better understanding of a_w lowering and prediction.

B. Investigating Alternative Humectants for Use in Foods

Reprinted on the following pages is a copy of the article published in Food Product Development, September 1975, pg.

INVESTIGATING ALTERNATIVE HUMECTANTS FOR USE IN FOODS

A. Elizabeth Sloan and Theodore P. Labuza

Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

1.   Introduction

Many hygroscopic chemical compounds are employed by the food industry to bind water in food products. Humectants are particulary important in the production of intermediate moisture foods. Various humectants such as polyols, sugars and salts are incorporated into these foods to lower the water activity (a_w) into the intermediate range. (a_w = 0.60--0.90) The increased shelf stability of these non-refrigerated IMF products is based on the principle that added solutes lower the availibility of water by binding it, thereby making it unavailable chemically and biologically. The solutes also increase the viscosity of the liquid phase thereby lowering reactant diffusion rates. The addition of solutes causes all reactions to decrease in rate as a_w is lowered. At some lowered water availibility the reactive solutes may crystallize out, thus stopping a reaction completely. This is the basic principle in many processing operations, such as drying.

Some of these humectants also exhibit other desirable effects as a result of their antimicrobial properties, texturizing characteristics, sweetening capacity and caloric value. At present due to increased cost and limited availibility of commonly used humectants alternatives must be found for use in food formulation. For example, the price of sucrose and propylene glycol, the main humectants used, has recently increased because of the world energy crisis. In order to successfully replace these two humectants, it is imperative that an accurate determination of the water sorption properties of all possible substitutes be made.

No direct comparison of the water binding properties of possible humectant compounds has ever been undertaken in the literature. This report deals with the water binding ability of several humectants as expressed by the water sorption isotherm.

Most of the literature available on the water sorption properties of humectants is very old and difficult to obtain. In addition, a great deal of the literature has been published in foreign language journals. In general, the articles available are poorly referenced as to methods used, contain many references to unpublished information and contain references to data which is unavailable, i.e. industrial confidential technical surveys. Much of the data which is presented here has been collected on a recent trip to Europe, through visits to various universities, institutes and confectionary company libraries concerned with research on the water sorption properties of humectants.

2. Humectants Under Study

Three general classes of chemical compounds are currently in use by the food industry as humectants, the polyols, sugars and salts. The polyols are the most desirable from a moisture sorption standpoint because of their low molecular weight and in some cases the fact that they are liquids. Based on a_w lowering ability, water holding capacity and low toxicity, propylene glycol, polyethylene glycol 400, glycerol, 1,3-butylene glycol and sorbitol are probably the most desirable polyols for use in food products. It should be noted that sorbitol, a sugar, is technically a polyol. On a similar basis glucose, sucrose and fructose are the most desirable sugars for use in food formulation. These are solid compounds at zero moisture content as is sorbitol. Lactose, due to its ready availibility and lower cost is also used but to a lesser extent. Owing to its minimal cost, excellent sweetening capabilities and its ready availibility corn syrup such as D.E. 42 is also a possible sucrose replacement. The literature on all these compounds was investigated. Two common salts, sodium chloride and potassium chloride were also surveyed as they are the oldest and most commonly used humectants.

All of the above humectants are on the GRAS list except 1,3-butylene

glycol. A petition however has been submitted to FDA to allow its use in pet foods while a petition to the GRAS list is also expected in the near future. Toxicological data for all the compounds are presented in Table 1.

3. The Sorption Isotherm

The water sorption isotherm represents an analysis of the water binding properties of a food material. As the equilibrium relative humidity or a_w (%ERH ÷ 100) increases from the dry state a humectant will hold or bind increasing amounts of water due to various physical factors. This relationship defines the moisture content of the system in equilibrium with the different values of a_w at constant temperature and is known as the sorption isotherm.

The physical – chemical factors responsible for a_w lowering have been reviewed by Labuza (1974), Labuza (1971), Labuza (1968), Heiss (1967) and Van Arsdel (1963).

4. Methodology Used in Humectant Isotherm Preparation

In order to determine a moisture sorption isotherm, both a_w and moisture content must be determined. Many methods have been employed to determine these two parameters, which are established at some constant temperature. The basis of the methods used have been discussed in detail by Toledo (1973) and Labuza (1974) and will not be presented here, however a brief description of technique is imperative.

5. Moisture Content Measurement

Due to the expense of equipment or difficulty in operation, the equilibration chamber method has been the most widely used method. This method is excellent if constant temperature can be maintained. Samples are placed in a closed chamber in which the a_w is controlled by saturated salt solution slurries, H_2SO_4-water solutions or glycerol-water mixtures. In a closed chamber these solutions give a constant a_w which varies very little with temperature. The moisture content of the samples after equilibration is then measured.

Table 1

TOXICOLOGICAL DATA

Substance	Route of Administration	Test Animal	LD_{50}
Propylene glycol	oral intragastric	rats	30.0g/Kg
1,3 - butylene glycol	subcutaneous intraperitoneal oral	rats rats rats	20.1 g/Kg 10.0 g/Kg 29.4 g/Kg
Polyethylene glycol 400	oral	rats	43.6 ml/kg
Sorbitol	intraperitoneal	mice (LD_{ca})	1.0 g/Kg
Glycerol	oral intravenous	mice mice	31.5g/Kg 7.56g/Kg
Sucrose	oral oral	rats-males rats-females	34.4g/Kg 29.7g/Kg
Fructose	intravenous	rabbits	15.0g/Kg
Lactose	intravenous	rabbits	1.5g/Kg
Glucose	intravenous	rabbits	35.0g/Kg
Corn Syrup	oral	rats	30.0g/Kg
Sodium Chloride	intraperitoneal	rats	5.0g/Kg
Potassium Chloride	intraperitoneal	rats (LD_{ca})	2.4 g/Kg

The gravimetric method is the most commonly used for determining moisture content. The weight change between the initial sample and the sample after equilibration is determined and the moisture content calculated. Of course, some absolute standard method for measuring water content is needed for defining dryness. For most humectants this method works quite well, however, problems resulting from evaporation of the glycols can occur and cause low moisture determination.

Some researchers have determined the moisture content of the samples using the Karl Fisher technique. The sample and the water reacts with a sulfite – iodine reagent which is titrated.

Many commercially available mechanical chambers have been used to provide a constant humidity. The better the accuracy the higher the cost of these chambers. Some of these chambers are equipped with an analytical balance so that moisture content of the sample can be determined continuously. As a chamber would be required for each a_w, this method is cost prohibitive and is seldom used.

Several special devices have been built in which the same sample can be exposed to a series of atmospheres of different humidities without removing it from the closed system. The Cahn RG Recording Electrobalance incorporated into a gravimetric adsorption apparatus and the McBain Quartz Spring Balance are examples. This latter method has been used primarily to measure the water sorption properties of lactose.

Scatchard et al (1938) have used an isopiestic technique to determine moist... content. In this technique samples are allowed to come to equilibrium through the vapor phase with a standard sulfuric acid – water mixture. Any change in weight is due to loss of solvent. The sulfuric acid mix is then titrated and its a_w related to a standard curve which will also be the a_w of the sample.

6. Direct a_w Measurement

Many techniques have been employed to measure the a_w of humectants. Electric hygrometers are commonly used. These devices contain sensors impregnated with salts.(LiCl or KCl) Water adsorbs on the sensor and causes a change in electrical resistance which is measured by a wheatstone bridge. A reference curve is prepared with constant humidity solutions to relate resistance to a_w. The sensor lifetime is short and is subject to contamination by volatile compounds which change the resistance. This device is not recommended for determining a_w's of food materials containing glycols, as they tend to adsorb onto the sensor and cause a reading error.

Norrish (1966) has designed an apparatus for measuring the a_w of confectionary solutions which consists of a hygrosensor in a dynamic system. Air is bubbled up through a sample bed and the % RH is measured by the hygrometer and recorded. The accuracy of this apparatus is limited.

A dew point technique has been employed by Money and Born (1951) and Grover (1940) in measuring the equilibrium relative humidity of sugar solutions. In this method the temperature of a surface in the air space in equilibrium with a sample is lowered until water condenses on it. The a_w is calculated from this temperature by use of a psychrometric chart. Sensitivity is based on the optical endpoint and is low.($\pm$ 4.0 % RH)

The direct measurement of the vapor pressure of water in the space surrounding the sample by manometric techniques is one of the best methods of a_w measurement. Manometric devices have been described by Taylor (1961), Karel and Nickerson (1964), and Labuza et al (1972). The vapor pressure manometer has been used by several researchers to measure the vapor pressure of humectant-water solutions. This system is very accurate ($\pm$0.002 a_w units) as long as the temperature remains constant. Another more complicated manometric technique was employed by Swan

(1926) for glycerol. The isoteniscope, another manometric device, has been used to measure the vapor pressure of saturated sugar solutions. (Whittier and Gould, 1930) This method is severely limited as it can only measure the vapor pressure of saturated solutions.

The a_w has also been calculated directly by measurement of freezing point depression. (Strong et al, 1970) Freezing point depression data for most aqueous humectant solutions is available in the International Critical Tables (1926) for the high a_w range. This method is based on the Clasius Clayperon equation for dilute systems using the calculated apparent concentration. The a_w can then be calculated using Raoult's Law. This method should only be applied for high a_w systems with low solute concentration.

7. Results and Discussion of Current Water Sorption Data

The data available for each particular class of compounds will be discussed collectively with specific references to individual humectants. The methods and conditions employed in the literature for the measurement of the humectant moisture sorption isotherms presented are listed in Table 2. All of the adsorption curves including those obtained in our laboratories were prepared by placing dry material in different equilibration chambers at increasing a_w's. Moisture content of the samples was determined after a specified period of time by the Karl Fisher technique, or by gravimetric analysis or by gas liquid chromatography after extraction with anhydrous methanol.

Most of the desorption systems were from measuring the a_w of solutions of different humectant/water ratios. However, the desorption curves prepared in our laboratory were also done by equilibrating a high moisture content sample over saturated salt solutions at lower a_w's.

8. Polyols

Very little data is currently available in the U.S. or in Europe on the water sorption properties of polyols, other than glycerol. Of the data found for propylene glycol (Figure 1) which behaves similarly to 1,3-butylene glycol,

TABLE 2

METHODS UTILIZED FOR HUMECTANT ISOTHERM DETERMINATION

Letter	Investigator	Method	Experimental Procedure	Temp °C	Days to Equilibration
A	Plitman, 1970	D	Electric Hygrometer Aqueous solution	25	---
B	Couvillion, 1972	D	Vapor Pressure Manometer, Aqueous soln.	RT	---
C	International Critical Tables, 1926	D	Calculation from freezing point depression aqueous solution	--	---
D	Celanese Chemcial Co., 1975	A	Equilibration chamber Karl Fisher Analysis	23	7
E	Labuza, 1971	D	VPM, aqueous solution	23	---
F	Grover, 1940	D	Dew Point Apparatus - aqueous solutions	20	---
G	Kowsooa	D	Electric hygrometer aqueous solution	NK	---
H	Norrish, 1966	D	Hygrosensor in Dynamic system - aqueous solution	20	---
I	Swan, 1926	D	Manometric Technique Aqueous solution	NK	---
J	Scatchard et al.	D	Isopiestic Technique	NK	---

D = desorption

A = adsorption

RT = Room Temperature

NK = not known

Table 2 continued

Letter	Investigator	Method	Experimental Procedure	Temp. °C	Days to Equili- bration
K	Cleland & Fetzer, 1944	A	Equilibration chamber- gravimetric analysis	25-30	7
L	ICI,United States,1973	D	Unknown, aqueous soln.	---	---
M	Heiss, 1955	A	Equilibration chamber- gravimetric analysis anhydrous sugar	20	60 (low) 12 (high)
N	Thieme, 1934	A	Equilibration chamber- gravimetric analysis	30	NK
O	ICI,United States 1975	A	Equilibration chamber- gravimetric analysis crystalline material	25	1-2mo.
P	Jenkins, 1960	D	Isoteniscope-aqueous solution	20	---
Q	Dittmar, 1935	A	Equilibration chamber- gravimetric analysis anhydrous sugar	25	NK
R	Whittier & Gould, 1930	D	Isoteniscope aqueous solution	25	---
S	Browne, 1922	A	Equilibration chamber- gravimetric analysis anhydrous sugars	20	25
T	Nelson, 1949	A	Equilibration chamber- gravimetric analysis anhydrous sugars	RT	NK
U	Mankower & Dye 1956	A	Equilibration chamber- gravimetric analysis amorphous sugars	25	800
V	Money & Born, 1951	D	Dew point technique aqueous solution	NK	---
W	Kargin,1957	A	Equilibration chamber- gravimetric analysis	20	NK
X	Sokolovsky, 1937	A	Equilibration chamber- gravimetric analysis Anhydrous sugars	20	40

Table 2 continued

Letter	Investigator	Method	Experimental Procedure	Temp °C	Days to Equili- bration
Y	CPC International Inc., 1974	A	Equilibration chambers - gravimetric analysis anhydrous sugars	20	9
Z	Lampitt & Bushill, 1931	A	McBain Quartz Spring Balance - amorphous sugar	25	9
A-1	Flink and Karel, 1972	A	Equilibration chambers- methanol -GLC moisture content analysis amorphous sugars	23	NK
A-2	Berlin, 1968	A	Cahn RG Recording Electrobalance	24.5	NK
A-3	Sloan & Labuza	A	Equilibration chamber gravimetric analysis	23	5-9mo.
A-4	Sloan & Labuza	D	VPM aqueous soln.	23	---
A-5	Sloan & Labuza	D	Equilibration Chamber methanol - GLC moisture content analysis	23	5mo.
A-6	Sloan & Labuza	D	Equilibration Chamber gravimetric analysis high a_w solutions	23	5mo.

Celanese (1975, ⊙) and ours (◻) represents the only adsorption data available, as humectant-water mixtures are generally considered to represent desorption systems.

The desorption curves prepared by various researchers appear to be fairly consistant below an a_w of 0.65. Couvillion's values (1972, ▲) and ours (△) were measured by the vapor pressure manometer (VPM). These most likely represent the actual desorption curve. Plitman's data, (1970, 0)was collected by use of an electric hygrometer. These readings show lower a_w values than those measured by the VPM at a_w's greater than 0.60. These lower values would be expected as the hygrometer is less accurate in the higher a_w range in samples containing glycols. Propylene glycol has a high vapor pressure.(0.05 mm Hg at 20°C) It can volatilize,adsorb onto the sensor and cause a reading error. As the VPM corrects for the additional vapor pressure contributed by the presence of the glycol, the VPM values are probably the most accurate. Although only two data points are available from calculation by freezing point depression (▨), they don't agree very well wtih the VPM measurements, but do fall on the curve for the hygrometer data. This calculation procedure is not very accurate at these lower a_w's due to the limitations inherent in the use of the Clasius Clayperon equation. Our desorption data (◉) using the equilibration chamber technique and GLC moisture measurement is inconsistent with the other desorption data. This phenomenon has not been explained.

Based on present knowledge of humectant water systems, it is unlikely that a hysteresis effect could occur with the use of these liquid glycols. However, the Celanese (1975, ●) and our data (◻) (Figure 1) both determined by an adsorption procedure, shows a lower moisture content at a given a_w than did the desorption curve. This pseudo-hysteresis effect can be the result of both evaporation of the polyol during the experiment and failure of the sample to reach final equilibrium if too short a time is used. The loss of glycol due to evaporation

FIGURE 1

THE WATER SORPTION PROPERTIES OF PROPYLENE GLYCOL
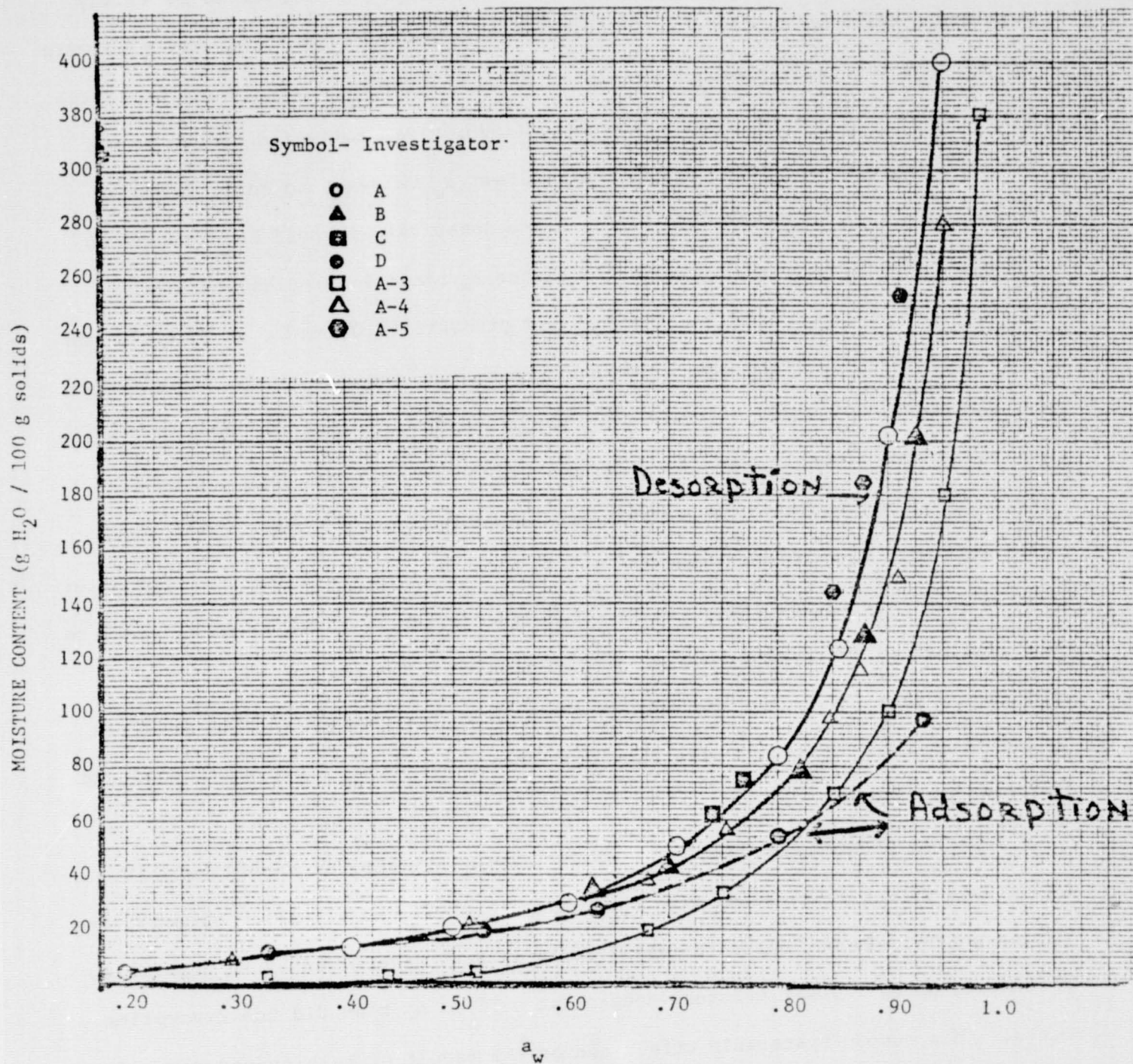
during the experiment is supported by data reported by Celanese (1975). This evaporation of glycol causes a particular problem when moisture content is calculated from the weight gain of the initial sample. As the loss of polyol would cause the sample weight gain to be less than the actual weight of the absorbed moisture, a lower moisture content would be calculated for a given a_w and thus a pseudo-hysteresis would occur.

In reference to Celanese's data (1975), the moisture content values were determined by the Karl Fisher method. This technique should account for the evaporation of the glycol as the final weight of glycol in the sample is used in the calculation of moisture content. However, as the samples were equilibrated for only 7 days, equilibrium most likely was not achieved. These two factors most likely account for the lower moisture content values exhibited by the adsorption system. Thus as a result of these factors, preparation of adsorption isotherms is extremely difficult.

The water sorption properties of glycerol (Figure 2) have been more extensively researched. This is most likely due to its wide-spread use in the confectionary industry. Our data for glycerol, which has a much lower vapor pressure than either propylene glycol or 1,3-butylene glycol. (0.002mm Hg at 20°C) does not exhibit a pseudo-hysteresis effect. As shown, the adsorption data (⊏) prepared by the equilibration chamber method (9 months) agree extremely well with the data prepared using the VPM and the desorption equilibration chamber method. As expected, the data collected by Cleland and Fetzer, (1944, ◆) and Celanese (1975, ○)show a lower moisture content at a given a_w. This is most likely due to the fact that these samples were kept in desiccators for only 7 days and have not reached equilibrium. Although a pseudo-hysteresis does occur for ' h vapor pressure polyols, glycols with a vapor pressure lower than that of glycerol (e.g. polyethylene glycol 400) will most likely not be effected.

Table 3 shows the moisture holding capacities of the polyol studies. Based

FIGURE 2

THE WATER SORPTION PROPERTIES OF GLYCEROL
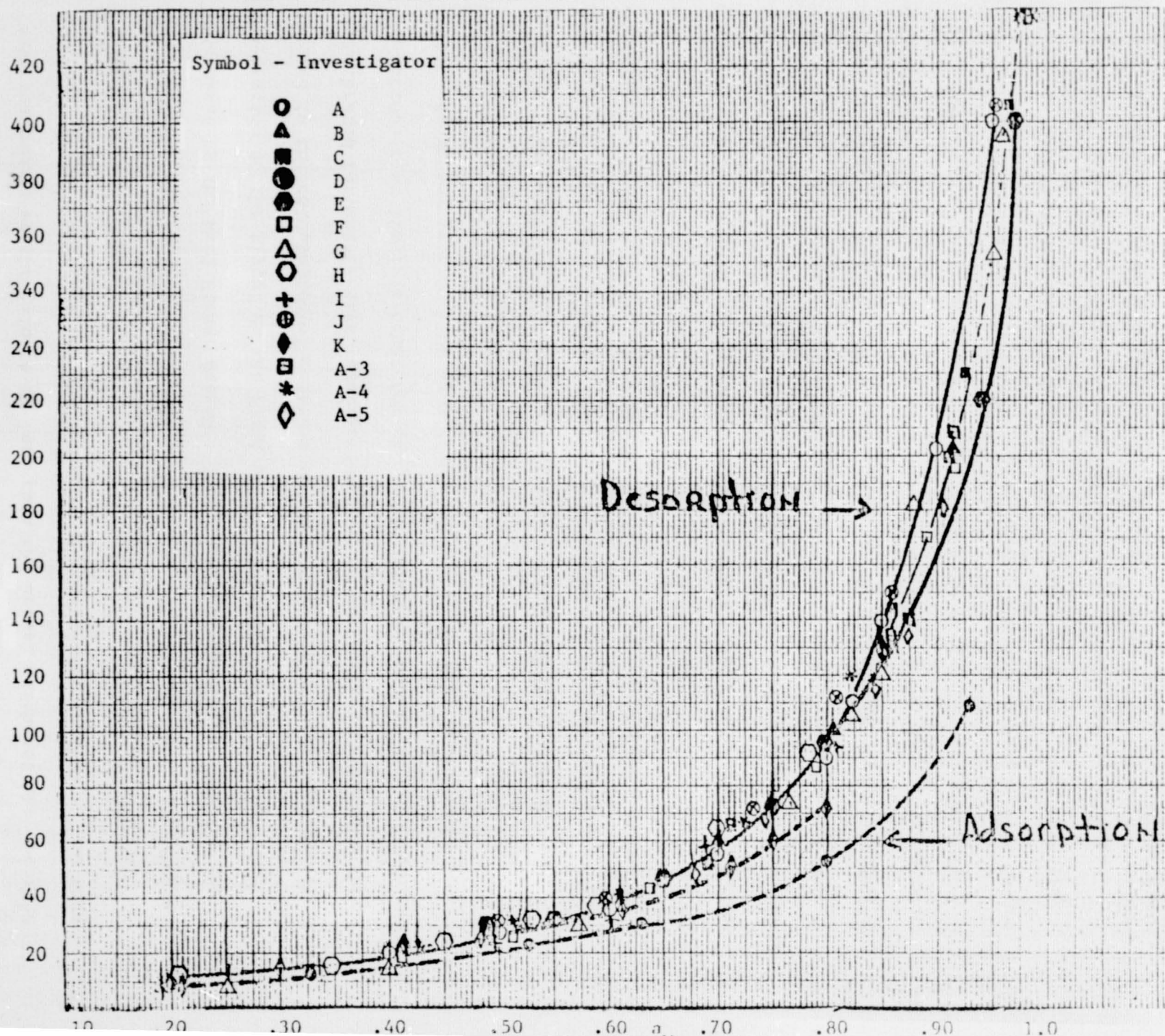
Table 3

WATER HOLDING CAPACITY OF POLYOLS*

Humectant	Moisture Content (g H_2O/100g solids)			
a_w =	0.60	0.70	0.80	0.90
1,3-butylene glycol	20.0	32.0	56.0	120.0
Propylene Glycol	30.0	46.0	52.0	148.0
Glycerol	38.0	57.0	96.0	160.0
Polyethylene Glycol 400	26.0	38.0	60.0	120.0

* In case of desorption system VPM values were considered to give the most accurate values.

solely on water holding capacity, glycerol seems to the most effective

humectant. The adsorption data was not used for the reasons discussed above.

Although propylene glycol and 1,3-butylene glycol are less hygroscopic than

glycerol they are still much better humectants than the sugars as will be seen.

9.   Sugars

Of the sugars studied, the water sorption properties of sucrose (Figure 3)

have been the most extensively researched. This is probably due to its wide-

spread use in the confectionary industry both in the U.S. and in Europe. A study

of the water sorption properties of sucrose is particularly important today due

to the very high cost for this commodity world wide. Figure 3 shows the available

data for sucrose to be divided into two distinct isotherms, with one having a

lower moisture content than the other. These isotherms may be explained by the

differences in the initial physical state of the sucrose which gives a pseudo-

sorption hysteresis effect. The curve showing the lower moisture content values

at a given a_w in Figure 3 comes from studies using anhydrous crystalline sucrose

subjected to an adsorption procedure. Our results ($\triangledown$,♦) are the only desorption

data available for crystalline sucrose. It shows that no significant difference

occurs between adsorption and desorption systems using crystalline sugar.

The upper curve (Figure 3) was found using

amorphous forms of sucrose made either from solution or from the dry state.

In general,   these dry samples were pretreated either by spray drying or freeze

drying. Although the data currently available on these amorphous forms is limited,

there appears to be no significant difference between the adsorption and desorption

data as could be expected. Thus the hysteresis that appears in Figure 3 is another

pseudo-effect based on initial physical form. It should be noted that one problem

in preparing adsorption isotherms of crystalline sugars is the rate of change

over to   the crystalline form. This is discussed in detail by Mankower and

Dye. (1956) This phenomenon makes it difficult to get a true isotherm value

at low a_w's. Similar curves as in Figure 3 were found for all the sugars.

FIGURE 3
THE WATER SORPTION PROPERTIES OF SUCROSE
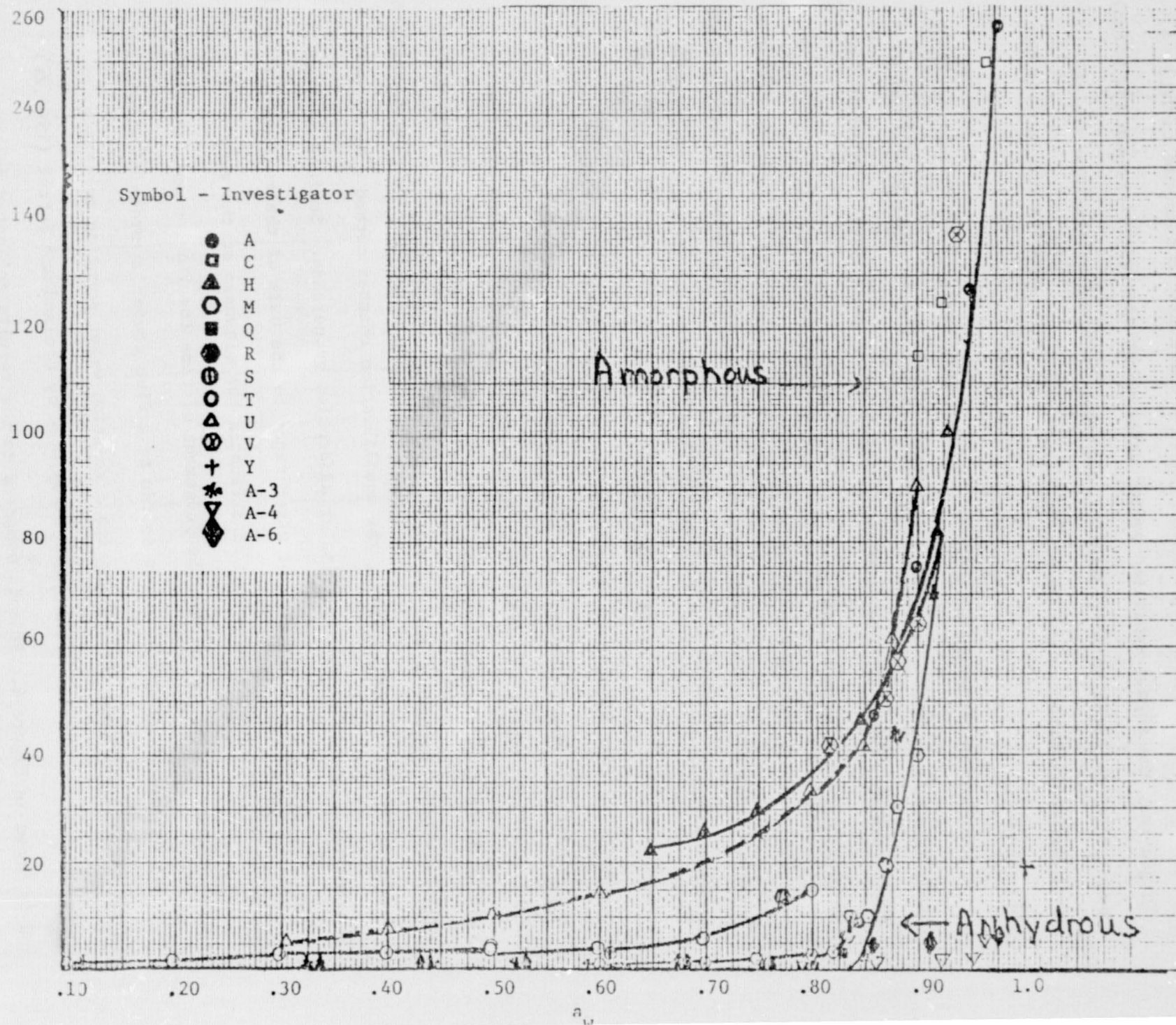
Water holding ability of these sugars as collected is compared in Table 4. In general, the sugars exhibited poorer water sorption properties than the polyols. Based soley on water holding capacity, sorbitol followed by fructose are the most desirable humectants. Sucrose has the third best capacity and lactose has the poorest.

All four of these sugars exhibit a pseudo-hysteresis effect resulting from differences in water holding ability of the crystalline and amorphous forms of these sugars. The amorphous form absorbs more water at a specific a_w than does the corresponding crystalline form.

Although the sugars are not as hygroscopic as the polyols, their excellent sweetening capacity and wide-spread use in the food industry make the need for an accurate determination of their water sorption properties even more apparent.

10. Corn Syrup

Although corn syrups are frequently incorporated into food products, especially in the confectionary industry, very little information is available on their water sorption properties. (Figure 4) The scarcity of data may have resulted in part at least from the nature of the materials. They are extremely viscous and noncrystalline throughout most of the humidity range. Such physical characteristics present difficulties when employing the equilibration chamber method coupled with gravimetric analysis to determine water adsorption properties. Surface sealing or skin formation stops the flow of water vapor to the bulk of the sample. As the final equilibrium is dependent on diffusion the slowness of the sample to reach constant weight may affect the judgement of time for final sample equilibration.

Drying and grinding corn syrup solids to a fine powder in order to eliminate these problems and then determining its adsorption water sorption properties by the equilibration chamber technique was employed by Cleland and Fetzer (1944, Δ) and is shown in Figure 4. This is compared to the results collected in our

-235-

TABLE 4

WATER HOLDING CAPACITY OF SUGARS & SALTS

Moisture Content (g H_2O/100g solids)

Sugars		Anhydrous				Amorphous		
	a_w = 0.60	0.70	0.80	0.90	a_w = 0.60	0.70	0.80	0.90
Sucrose	3.0	5.0	10.0	---	14.0	20.0	35.0	65.0
Glucose	1.0	3.5	7.5	12.5	1.0	3.5	8.0	22.0
Fructose	14.0	22.0	34.0	47.0	18.0	30.0	44.0	80.0
Lactose	.01	.01	.05	.10	4.5	4.7	4.7	---
Sorbitol	17.0	22.0	37.0	76.0	25.0	35.0	55.0	110.0
Corn Syrup	----	----	----	----	14.0	20.0	30.0	54.0

Salts								
NaCl (adsorption)	0.1	0.1	130.0	585.0	---	---	---	---
NaCl (desorption)	---	---	385.0	590.0	---	---	---	---
KC1 (adsorption)	0.1	0.1	0.1	0.1	---	---	---	---
KC1 (desorption)	---	---	0.1	580.0	---	---	---	---

FIGURE 4
THE WATER SORPTION PROPERTIES OF CORN SYRUP

Symbol – Investigator

Symbol	Investigator
△	K
▣	A-3
○	A-4
◉	A-6

MOISTURE CONTENT (g H_2O / 100 g solids)

a_w
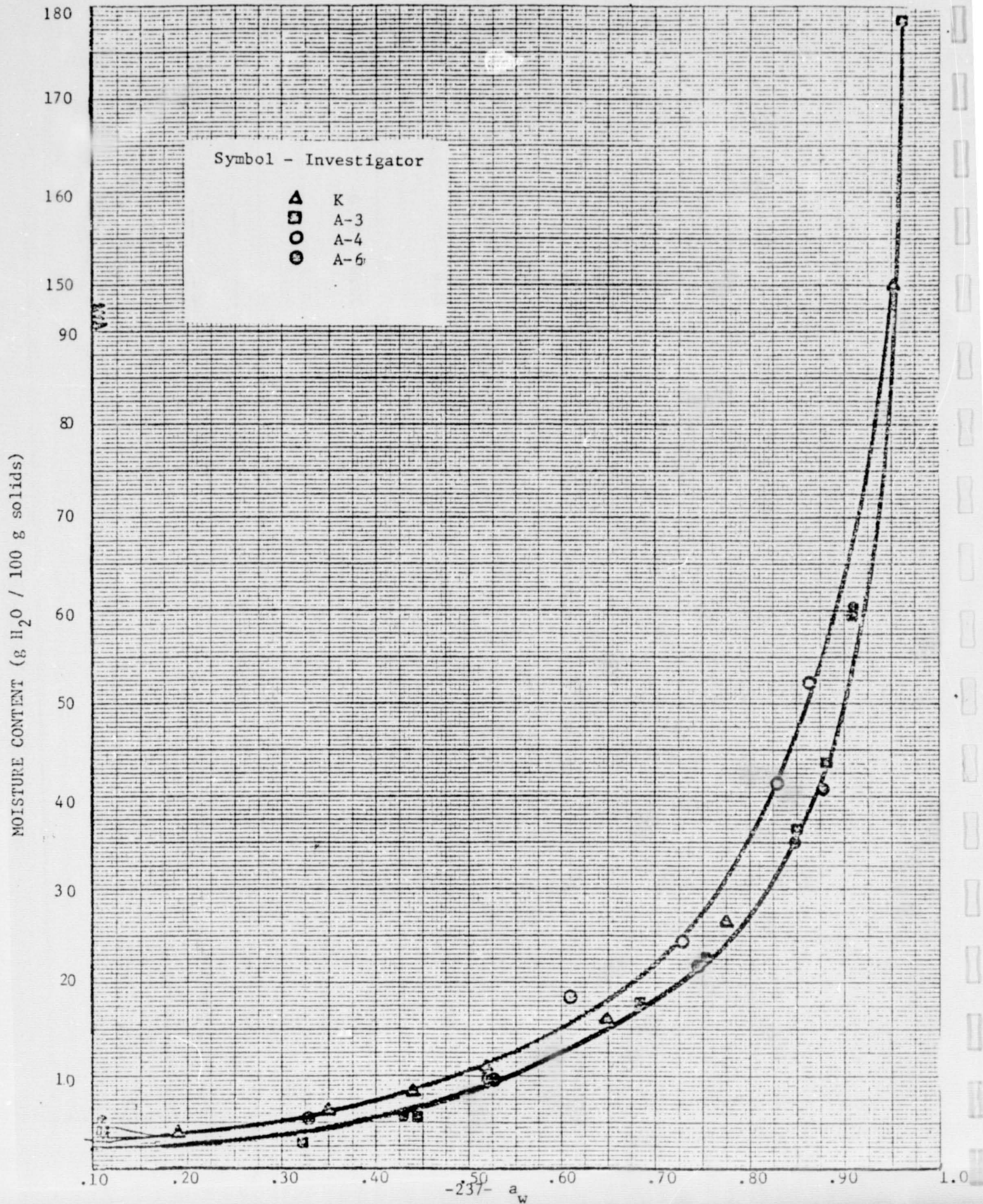
laboratory. As seen no true hysteresis occurs. The VPM results give a lower a_w than the desiccator methods at the same moisture content. This difference however is small. As seen in Table 4, the corn syrup solids have a water binding capacity close to that of sucrose.

11. Salts

Although NaCl is probably the oldest and most commonly used food ingredient, very little information is available on its water sorption properties. (Figure 5) The only adsorption data available on NaCl is our data ($\blacksquare$). Figure 5 shows desorption data collected for water – NaCl mixtures up to the saturation point. The measured values determined by the VPM method are believed to be the most accurate desorption data available. As expected a sorption hysteresis effect occurs probably due to supersaturation. As seen in Table 4, compared to the polyols and the sugars in aqueous solutions in the high range of a_w, NaCl and KCl appear, to be a superior humectants. This increased a_w lowering ability exhibited by the salts may be explained by the smaller molecular weight which increases its ability to bind or structure more water.

12. Conclusions

Polyols are the most desirable humectants from a water holding standpoint. However, in order to meet the criteria for a good replacement for propylene glycol or sucrose and to minimize the cost, a combination of the humectants presented here will be necessary to achieve the desired goal. The isotherms for all of these humectants are available upon request.

FIGURE 5
THE WATER SORPTION PROPERTIES OF NACL
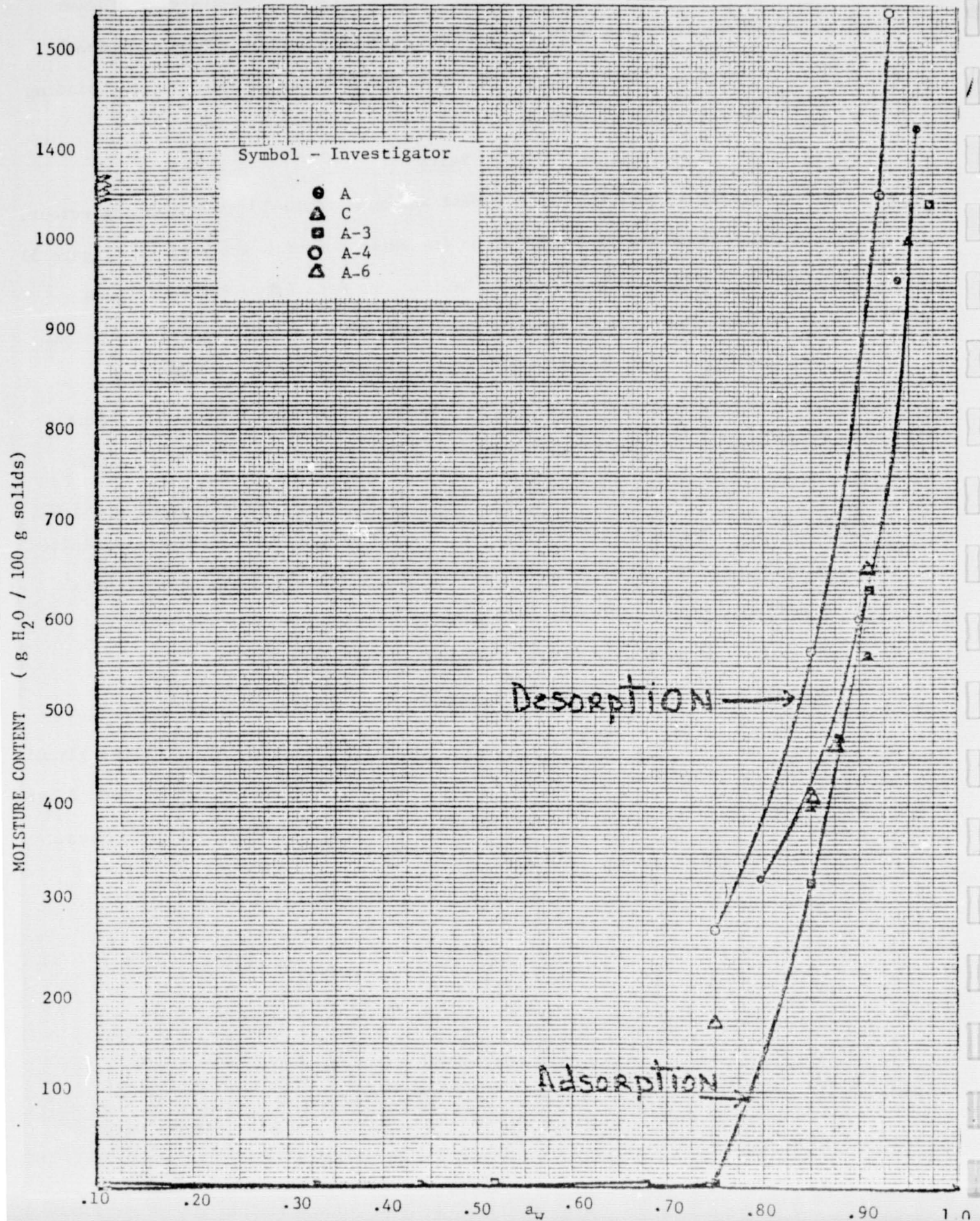
Berlin, E., Anderson, B.A., Pallansch, M.J. Water Vapor Sorption Properties of Various Dried Milks and Wheys. J. Dairy Sci. 51:1339. 1968a.

Berlin, E., Anderson, B.A., Pallansch, M.J. Comparison of Water Vapor Sorption by Milk Powder Components. J. Dairy Sci. 51:1912. 1968b.

Browne, C.A. Moisture Absorptive Power of Different Sugars and Carbohydrates under Varying Conditions of Atmospheric Humidity. J.Ind. & Eng. Chem. 14:712. 1922

Celanese Chemical Co. "1,3 -Butylene Glycol - Comparative Hygroscopicity Data". Celanese Chemical Co., New York, New York.

Celanese Chemical Co. "1,3-Butylene Glycol (S-26-10)" Celanese Chemical Co. New York, New York.

CPC International Inc. "Cerelose - Dextrose, A Highly Versitile Food Ingredient". CPC International Inc., Englewood Cliffs, New Jersey.

Cleland, J.E., Fetzer,W.R. Moisture Absorptive Power of Starch Hydrolyzates. Ind. & Eng. Chem. 36:552. 1944.

Couvillion,J.L. Preservation of Foods with 1,3 -butylene glycol. Celanese Chemical Co. Technical Report JLC-51-72. April, 1972.

Dittmar, J.H. Hygroscopicity of Sugars and Sugar Mixtures. Ind. Eng. Chem. 27:333. 1935.

Flink,J., Karel,M. Mechanism of Retention of Organic Volatiles in Freeze-Dried Systems. Food Tech. 7: 199. 1972.

Grover, D.W., Nicol,J.M. The Vapor Pressure of Glycerin Solutions at 20°C. J. Soc. Chem. Ind. 59:175. 1940.

Heiss, R. Haltarbeit und Sorptionsverhalten Wasserarmen Lebensmittel. Springer-Verlag, Berlin. 1967.

Heiss, R. Versuche Uber Feuchtigkeitsempfindlichkeit und verpackung von Puderzucker. Verpackungs - Rundschau. 12: 1. 1961.

ICI, United States Inc. Equilibrium Hygroscopicity of Sorbitol. ICI, United States, Inc., Chicago, Illinois. 1973.

ICI, United States Inc. Personal Communication - Leslie K. Swanson (Technical Correspondant). ICI, United States, Inc., Chicago, Illinois. 1975.

International Critical Tables. International Critical Tables. Vol. III. McGraw-Hill, New York. 1926.

Jenkins, V.F. Unpublished Data. Howards of Ilford Ltd., England. 1960.

Karel, Nickerson,J.T.R. Effects of Relative Humidity, Air and Vacuum on Browning of Dehydrated Orange Juice. Food Tech. 18:104. 1964.

Kargin, See Stolle et al.

Kowsooa, _____. Preparation and test of Glycerol Solutions for Control of Relative
    Humidity. Source Unknown.

Labuza, T.P. Sorption Phenomenon in Foods. Food Tech. 22:15. 1968.

Labuza,T.P. Analysis of Storage Stability of Intermediate Moisture Foods.
    NASA Final Report, NAS 9-10658. pg.158. 1971.

Labuza,T.P. Properties of Water as Related to the Keeping Quality of Foods
    Proc. 3rd Int'l Congress Food Science & Technology. pg. 618. 1971.

Labuza,T.P.,McNally,L.,Gallagher, D.,Hawkes, T.,Jurtado,F. Stability of
    Intermediate Moisture Foods. J. Food. 37:154. 1972.

Labuza, T.P. Theory, Determination and Control of Physical Properties of Food
    Materials. D. Reidel Publishing Co., Dordrecht, Holland. Chapter 10,
    pg. 119. 1974.

Labuza, T.P., Rutman,M. Effect of Surface Active Agents on Sorption Isotherms
    of a Model System . Can. J. Chem. Eng. 46:364. 1968.

Labuza,T.P.,Simon,I.Surface Tension of Fruit Juices. Food Tech. 23:96. 1969.

Lampitt, L.H.,Bushill,J.H., The Physico-Chemical Constitution of Milk Power.
    Analyst 56:178. 1931.

Mankower,B.,Dye,W.B. Equilibrium Moisture Content and Crystallization of
    Amorphous Sucrose and Glucose. Agric. and Food Chem. 4:72. 1956.

Money,R.W.,Born, R. Equilibrium Humidity of Sugar Solutions. J. Sci. Food Agric.
    2:180. 1951.

Nelson,T.J. Hygroscopocity of Sugar and Other Factors Affecting Retention of
    Quality. Food Tech. 3:347. 1949.

Norrish,R.S. An Equation for the Activity Coefficients and Equilibrium Relative
    Humidities of Water in Confectionary Syrups. Brit. J. Food Tech. 1:25.
    1966.

Norrish,R.S. Selected Tables of Physical Properties of Sugar Solutions. BFMIRA
    Scientific and Technical Survey #51. July, 1967.

Plitman,M. Viability of Staphylococcus aureus in Intermediate Moisture Foods.
    M.S. Thesis. Massachusetts Institute of Technology. Cambridge, Massachusetts.
    1973.

Scatchard,G.,Hamer,W.J., Wood,S.E., Isotonic Solutions. I. The Chemical Potential
    of Water in Aqueous Solutions of Sodium Chloride, Potassium Chloride,
    Sulfuric Acid, Sucrose, Urea and Glycerol at 25°C. J. Am. Chem. Soc.
    60:3061. 1938.

Sokolovsky,A. Effect of Humidity on Hygroscopic Properties of Sugars and Caramel.
    Ind. Eng. Chem. 29:1422. 1937.

Strolle,E.O.,Cording,J.R., McDowell,P.E., Eskew,R.K., Effect of Sucrose on
    Crispness of Explosion-puffed Apple Pieces Exposed to High Humidities.
    J. Food Sci. 35:338. 1970.

Strong,D.H.,Foster,E.,Duncan,C. Influence of a_w on the Growth of Cl. perfringens.
    Applied Microbiol. 19:980.1970.

Swan,E. The Deliquescent Properties of Magnesium Chloride, or Calcium Chloride
    and of Glycerol. J. Textil. Inst. 17:517. 1926.

Taylor,A.A. Determination of Moisture Equilibrium in Dehydrated Foods. Food
    Tech. 15:536. 1961.

Thieme, J.C. Th Hygroscopic Properties of Raw Sugars and Molasses. Int. Sugar
    J. 36:192. 1934.

Toledo,R.T. Determination of Water Activity in Foods. Proc. Meat Ind. Res. Conf.
    AMIF.March,1973.

Van Arsdel,W.B. Food Dehydration. AVI Publishing Co. Westport, Conn. 1963.

Whittier,E.O.,Gould,S.P. Vapor Pressures of Saturated Equilibrated Solutions of
    Lactose, Sucrose, Glucose and Galactose. Ind. & Eng. Chem. 22:77. 1930.

C. Effect of Order-of-Mixing on a_w Lowering Ability of Food
Humectants

Reprinted on the following pages is a copy of the article
submitted to the Journal of Food Science for publication. The paper
was presented at the 35th Annual Meeting of the Institute of Food
Technologists.

EFFECT OF ORDER-OF-MIXING ON A_W LOWERING

ABILITY OF FOOD HUMECTANTS

A.E. Sloan, P.T. Waletzko and T.P. Labuza

Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

1. ABSTRACT

The effect of the order-of-mixing on four humectants of major concern to the U.S. pet food industry was investigated using a meat-soy flour model intermediate moisture dog food system. Humectants were added dry or as a solution and the resultant water activities were measured. Both adsorption and desorption curves for pure humectant/water systems were prepared for each humectant. It was shown that even though the pure humectant isotherm may exhibit a hysteresis effect, there is no significant difference in the final a_w achieved as a result of the order-of-mixing in the a_w range of intermediate moisture food products.

2. INTRODUCTION

The successful introduction of intermediate moisture food (IMF) processing into the world pet food markets has results in an increased interest in this technology (Rhodes, 1975). Intermediate moisture food products have a moisture content of 15-40% water and a water activity (a_w) range of about 0.65 to 0.90. They require no rehydration and have a soft plastic texture. Intermediate moisture foods are shelf stable and can be formulated to meet specific nutrients needs (Smith and Norvell, 1975).

Various humectants, or water binding agents such as polyols, sugars and salts are incorporated into these products to lower the a_w into the intermediate moisture range (Bone, 1973, Bone et al., 1974). The increased shelf stability of these non-refrigerated IMF products is based on the principle that added solutes lower the availability of water by binding it, thereby making it unavailable chemically and biologically. The solutes also increase the viscosity of the liquid phase, thereby lowering reactant diffusion rates (Heiss, 1967; Van Arsdel, 1963; Labuza, 1968; Labuza, 1971; Labuza, 1974a). In addition to their ability to bind water, some humectants also exhibit other desirable effects in a food system as a result of their antimicrobial properties (Labuza, 1974b; Plitman, 1973; Celanese, 1971; Patsch, 1969, U.S. Patent 3,806,615) texturizing characteristics (Celanese S-26-10; Livengood, 1970) sweetening capacity and caloric value (Doherty, 1972).

Semi-moist pet foods represent the majority of IMF food products now in the marketplace. Semi-moist pet foods account for close to 40%

of the U.S. pet food market (Rhodes, 1975). A typical soft moist dog food formulation is shown in Table 1 (Bone, 1969).

The humectants used in this particular formulation are sucrose, propylene glycol, sorbitol and salt. In this case sucrose is the main humectant used at a level of 22% by weight. The other 3 humectants constitute a minor portion by weight of the product.

Humectants can be added into a food system either in a dry state or as a solution by previously dissolving the humectant in water. As a result of the method of addition of water into a food system, i.e. adsorption vs. desorption, a hysteresis effect may occur (Rao, 1941; Taylor et al., 1961; Bettleheim and Ehrlich, 1963; Berlin et al., 1969; Mackenzie and Luyet, 1971). Wolfe et al. (1972) also showed that a hysteresis effect can result in many foods especially those food products high in sugar. A hysteresis effect is one in which the amount of water which can be held in a food at a given a_w value differs depending on the method of addition of the water into the food, i.e. an adsorption vs. a desorption procedure, with the desorption procedure holding more water at a given a_w.

The reasons for hysteresis have been discussed in detail by Labuza, 1974; Labuza, 1968; Labuza and Rutman, 1968, Gregg and Sing, 1967). The effect may be due to the structural effect of pores as described by Rao's theory (Rao, 1941) and shown by Labuza and Simon (1969). Two other factors which can contribute to hysteresis are supersaturation of solutes in the water of the food during desorption and the physical state of these solutes (Labuza, 1974; Sloan and Labuza, 1975). Supersaturation can occur when the food is dehydrated. If the rate of water removal is rapid the viscosity may increase too rapidly for crystallization to take place.

TABLE 1

TYPICAL COMPOSITION OF SOFT MOIST DOG FOOD

Ingredient	Per cent
Meat-by-products	32.0
Soy flakes	33.0
Sugar	22.0
Skimmed dry milk	2.5
Calcium and phosphorus	3.3
Propylene glycol	2.0
Sorbitol	2.0
Animal fat	1.0
Emulsifier	1.0
Salt	0.6
Potassium sorbate	0.3
Minerals, vitamins and color	0.3

The resulting solution holds more water than the corresponding crystalline form. In addition the supersaturation state may form an amorphous glass (White and Cakebread, 1966; Parks and Huffman, 1928). The third effect is related to this same phenomenon namely, crystalline material absorbs less water per unit weight basis than does amorphous material because less hydrophilic sites are exposed to the vapor phase (Mankower and Dye, 1956). Thus, it is possible that there would be a smaller degree of a_w lowering by crystalline solutes if they are added dry to a food system (an adsorption process) as compared to adding the solutes by first dissolving them in water, which could be considered a desorption process.

This hysteresis or order-of-mixing phenomenon was investigated in a model semi-moist dog food system for the four humectants used in the dog food formulation (Table 1) as these are the humectants of major concern to the pet food industry.

3. MATERIALS AND METHODS

The available literature was surveyed to determine if a hysteresis effect occurred for pure humectant/water systems. In addition both adsorption and desorption isotherms were measured for each humectant by static moisture gain from dry material using amorphous and/or crystalline material or by moisture loss from a liquid solution. A series of saturated salt solution desiccators were used for periods of from 4-8 months at room temperature. In addition, desorption curves were prepared by measuring the a_w of humectant/water solutions by the vapor pressure manometric technique (Labuza, 1974). The humectant curves and procedures are discussed in detail by Sloan and Labuza, (1975). The curves may be obtained from the authors.

The four humectants investigated in this study and their suppliers are listed in Table 2. The procedure used to investigate the order-of-mixing phenomenon for the four major humectants used in IMF pet foods is shown in Figure 1. A meat-soy flour model IMF dog food system was employed. The composition of this model system is listed in Figure 1. Lean ground beef, soy flour (20 PDI - Cargill, Inc., Minneapolis, MN) citric acid (T.J. Baker Chemical Co.) and potassium sorbate (Annheuser-Busch, Inc., St. Louis, MO) were ground in 50g portions in an osterizer for 3 minutes at high speed. These portions were combined in a large stainless steel bowl, hand mixed and equilibrated in a covered 5 lb. glass jar. After equilibration the mixture was divided into 2 equal portions. In order to reach a given a_w, for example 0.90, a predetermined amount of water was mixed into one portion in a Brabender-Farinograph bowl, mixed at high speed for 5 minutes and re-equilibrated. The amount of water necessary to obtain a particular a_w was determined by preparing a moisture sorption isotherm of the meat-soy flour model system by adding increasing increments of water to the product and measuring the resultant a_w by the vapor pressure manometric technique (Labuza, 1974).

The remaining dry portion was subdivided into 6 equal portions. To each one of these 6 portions, the same amount of water necessary to reach 0.90 was added. In this water, different amounts of the humectants shown in Table 2 were dissolved prior to their addition to the meat-soy flour system. This constituted the "wet mix" system. All humectants were used as purchased. Amorphous sucrose was prepared by freeze-drying a 25% w/w solution of crystalline sucrose for 48 hr. Each portion was subdivided into 3 equal parts and sealed in 202 x 214 cans. After an 18 hr equilibration period, one can per humectant concentration was

TABLE 2

Humectant	Supplier
Propylene glycol	Dow Chemical, #156993, Midland, Mich.
Sucrose, (crystalline)	Mallincrodt, #8360, St. Louis, MO.
Amorphous sucrose	25% freeze-dried solution of crystalline sucrose (w/w)
Sorbitol (crystalline)	Atlas Chemical Div., ICI United States, #573', Lot 542D, Chicago, IL
Sodium chloride	Mallinckrodt, #54290, St. Louis, MO.

FIGURE 1

PROCEDURE USED TO INVESTIGATE ORDER OF MIXING PHENOMENON

COMPOSITION: % by wt.

	% by wt.
MEAT	19.5
SOY FLOUR	78.2
CITRIC ACID	2.0
K-SORBATE	0.3
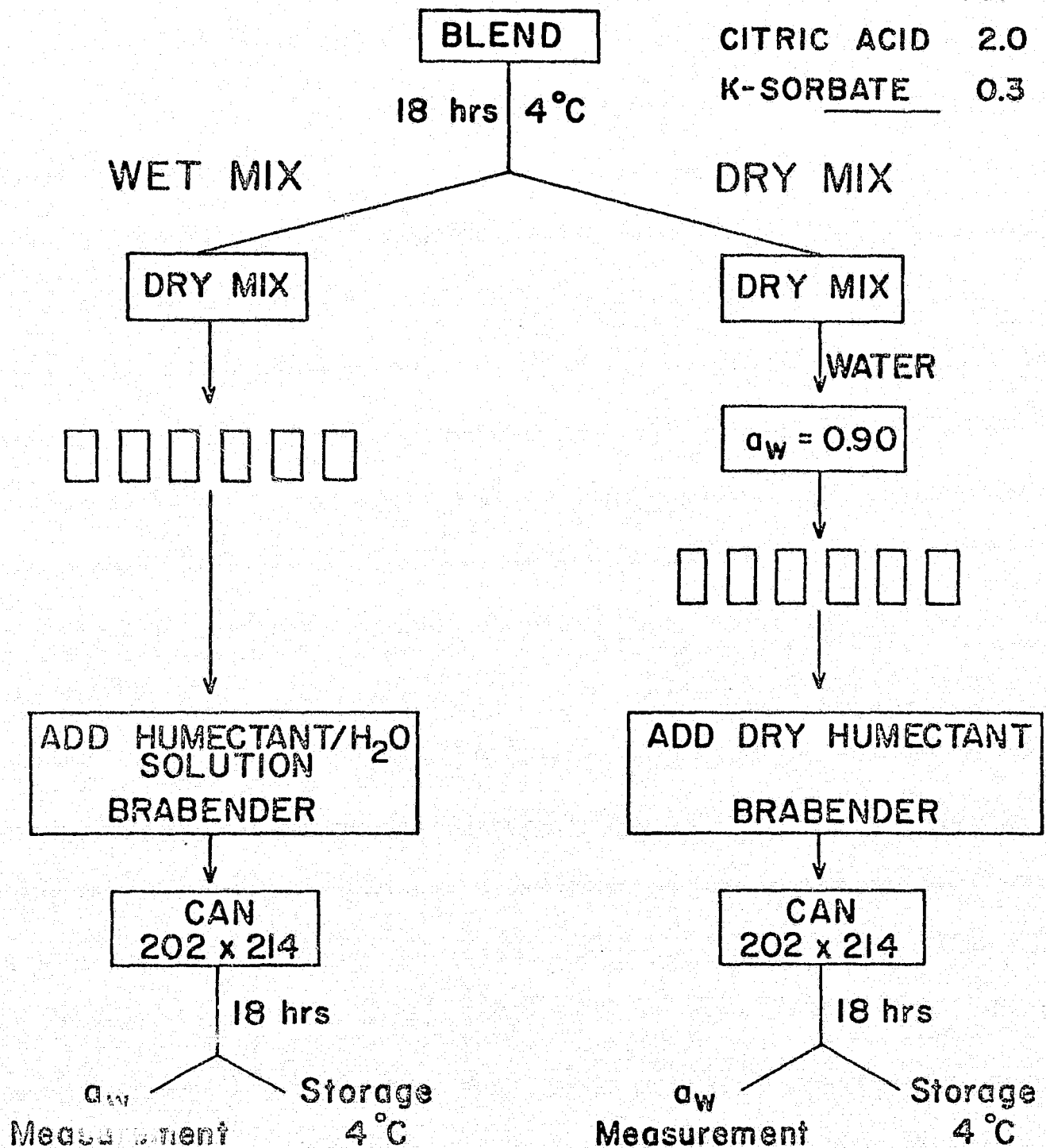
removed and its a_w measured by the vapor pressure manometric technique. The remaining cans were stored at 4°C. The a_w was also measured after storage for various time periods.

After the initial re-equilibration period for the remaining pre-moistened initial mixture, increasing increments of humectants were added dry into the appropriate amount of system with an initial a_w of 0.90. This is the "dry mix" portion. The same procedure was followed for equilibration and measurement.

As it was felt that it would be more advantageous to have a larger sample size for a_w measurement, the Fett-Vos isopiestic technique for measurement of a_w was employed (Fett, 1973; Vos and Labuza, 1974).

4. RESULTS AND DISCUSSION

Figure 2 shows the sorption curve for propylene glycol as found in the literature. The references and methods used are listed in Table 3. Although this data shows a hysteresis effect, the adsorption curves do not give a true indication of the water sorption properties, due to the occurence of evaporation of the sample during equilibration in desiccators, at constant humidity. This effect was also demonstrated by another high vapor pressure polyol studied, 1,3 butylene glycol. During the equilibration period in the desiccators an undetermined amount of glycol in the sample is continually being lost (Sloan and Labuza, 1975). Thus, a true equilibrium condition can never be reached. Secondly, in most studies of humectants not enough time was allowed for the samples to reach true equilibrium. As shown by our data for glycerol (Figure 3) which was equilibrated for 9 months at 23°C, no hysteresis occurs when comparing adsorption, desorption and VPM data. Glycerol has a much lower vapor pressure and

FIGURE 2

THE WATER SORPTION PROPERTIES OF PROPYLENE GLYCOL
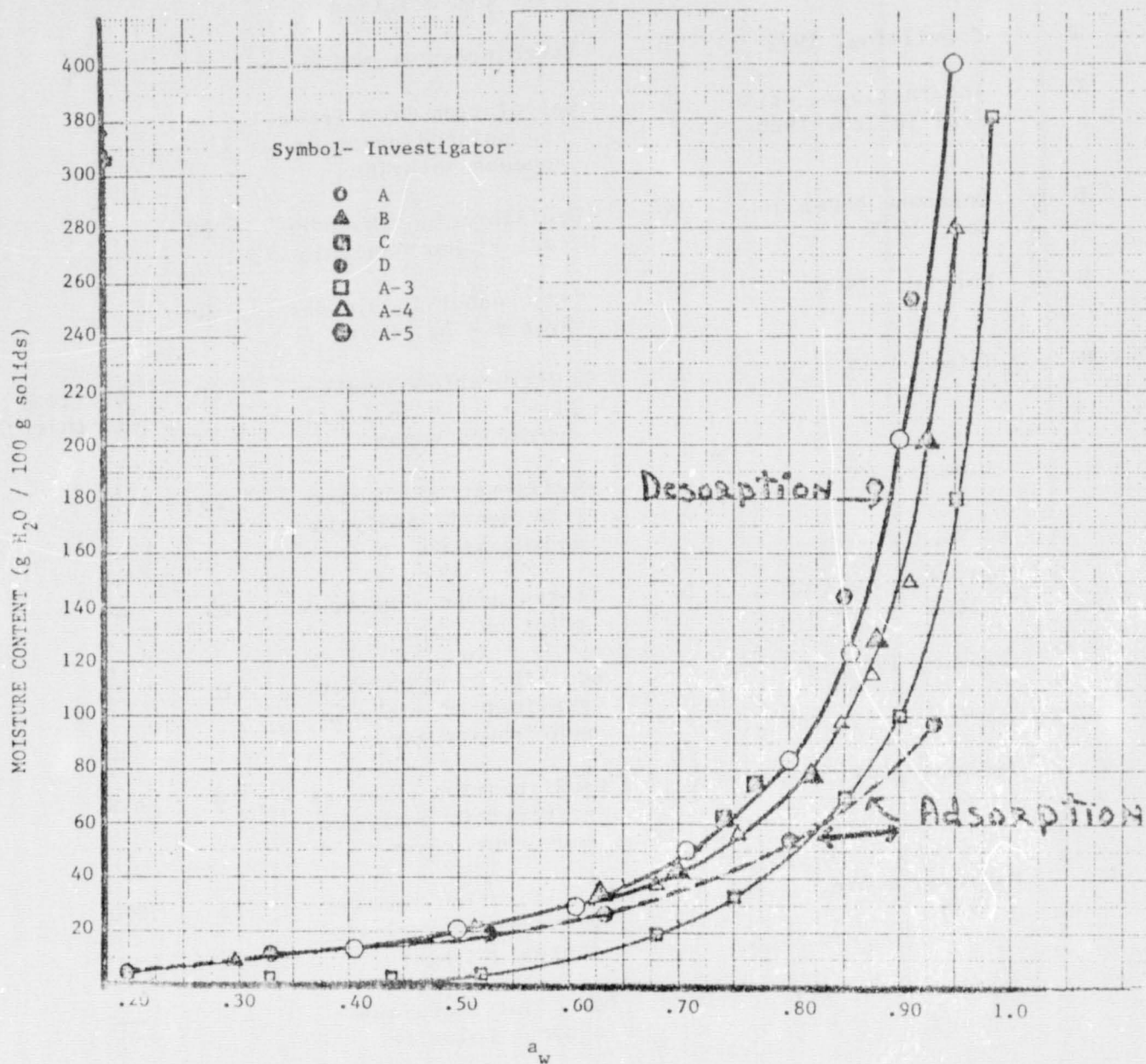
TABLE 3

METHODS UTILIZED FOR HUMECTANT ISOTHERM DETERMINATION

Letter	Investigator	Method	Experimental Procedure	Temp. °C	Days to Equilibration
A	Plitman, 1970	D	Electric hygrometer aqueous solution	25	—
B	Couvillion, 1972	D	Vapro pressure manometer	RT	—
C	International Critical Tables, 1926	D	Calculation from freezing point depression – aqueous solution	—	—
D	Celanese Chemical Co., 1975	A	Equilibration chambers – Karl Fisher Analysis	23	7
H	Norrish, 1966	D	Hygrosensor in Dynamic System – Aq. Soln.	20	—
M	Heiss, 1955	A	Equilibration chambers – gravimetric analysis anhydrous sugar	20	60 (low) 12 (high)
Q	Dittmar, 1935	A	Equilibration chamber – gravimetric analysis anhydrous sugar	25	—
R	Whittier and Gould, 1930	D	Isoteniscope – aqueous solution	25	—
S	Browne, 1922	A	Equilibration chamber – gravimetric analysis anhydrous sugar	20	25
T	Nelson, 1949	A	Equilibration chambers – gravimetric analysis anhydrous sugar	20	N.K.
U	Mankower & Dye, 1956	A	Equilibration chamber – gravimetric analysis amorphous sugar	25	800
V	Money & Born, 1951	D	Dew point technique aqueous solution		—

Y	CPC International Inc.	A	Equilibration chambers – gravimetric analysis anhydrous sugars	20	9
A-3	Sloan and Labuza, 1975	A	Equilibration chambers – gravimetric analysis	23	5--9 mo.
A-4	Sloan and Labuza, 1975	D	Vapor pressure manometer	RT	----
A-5	Sloan and Labuza, 1975	D	Equilibration chamber – GLC analysis	23	5 mo.
A-6	Sloan and Labuza,	D	Equilibration chamber – gravimetric analysis high a_w solutions	23	5 mo.

D = desorption

A = adsorption

RT = room temperature

NK = not known

FIGURE 3

H₂O Sorption Properties of Glycerol
AT 35°C (SLOAN AND LABUZA, 1975)

O – ADSORPTION
DESORPTION
A – DESICCATOR
⊙ – VPM
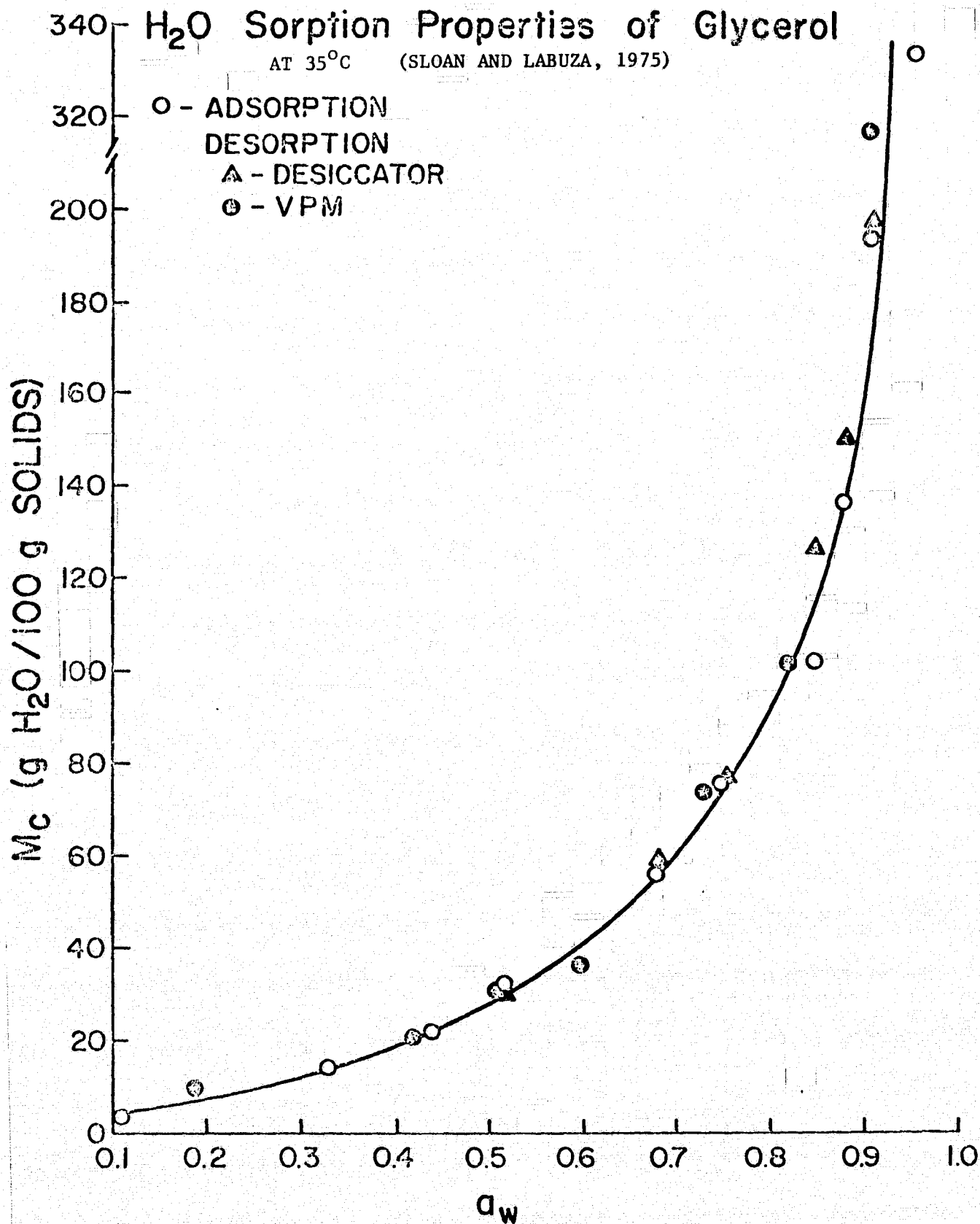
should not evaporate to any significant extent during equilibration.
The same was found for polyethylene glycol 400. Thus, it can be presumed
that liquid humectants such as propylene glycol should not show a hysteresis
effect with water since there is no crystalline-amorphous transition.

The water sorption isotherm for sucrose is shown in Figure 4. A
true sorption hysteresis occurs which is dependent on the initial crystalline
form of the material as was expected. The same sorption curve is exhibited
by amorphous sucrose for either adsorption of water by predried material,
desorption from a solution or from a_w measurement of prepared liquid
solutions.

The isotherms for sorbitol as well as other sugars and the two salts
studied NaCl and KCl also exhibited the same effect. The hysteresis
range for the crystalline materials used in this study was (1) for
sucrose 0.3 to 0.92 (2) for sorbitol 0.5 to 0.97 and (3) for sodium
chloride 0.75 to 0.95. Thus it should be expected that if an order-of-
mixing effect occurs, it would be manifested in the IMF range.

Table 4 shows the resultant a_w's after the addition of propylene
glycol to the meat-soy flour model system using both a wet and dry mix
procedure. As expected, the order-of-mixing of this humectant has no
effect on the degree of a_w lowering, since no true hysteresis was found
for the water/humectant system. Thus, the method of addition of glycol
in the food process should make no difference. In addition there is little effect of
storage.

The results obtained for crystalline and amorphous sucrose are
shown in Table 5. It should be remembered that a true sorption hysteresis
existed for sucrose between a_w = 0.30 to 0.92. As shown the order-of-
mixing of anhydrous crystalline sucrose does not have an effect on the
resultant a_w even at high concentration. Thus, in the dry mix system,

FIGURE 4

THE WATER SORPTION PROPERTIES OF SUCROSE
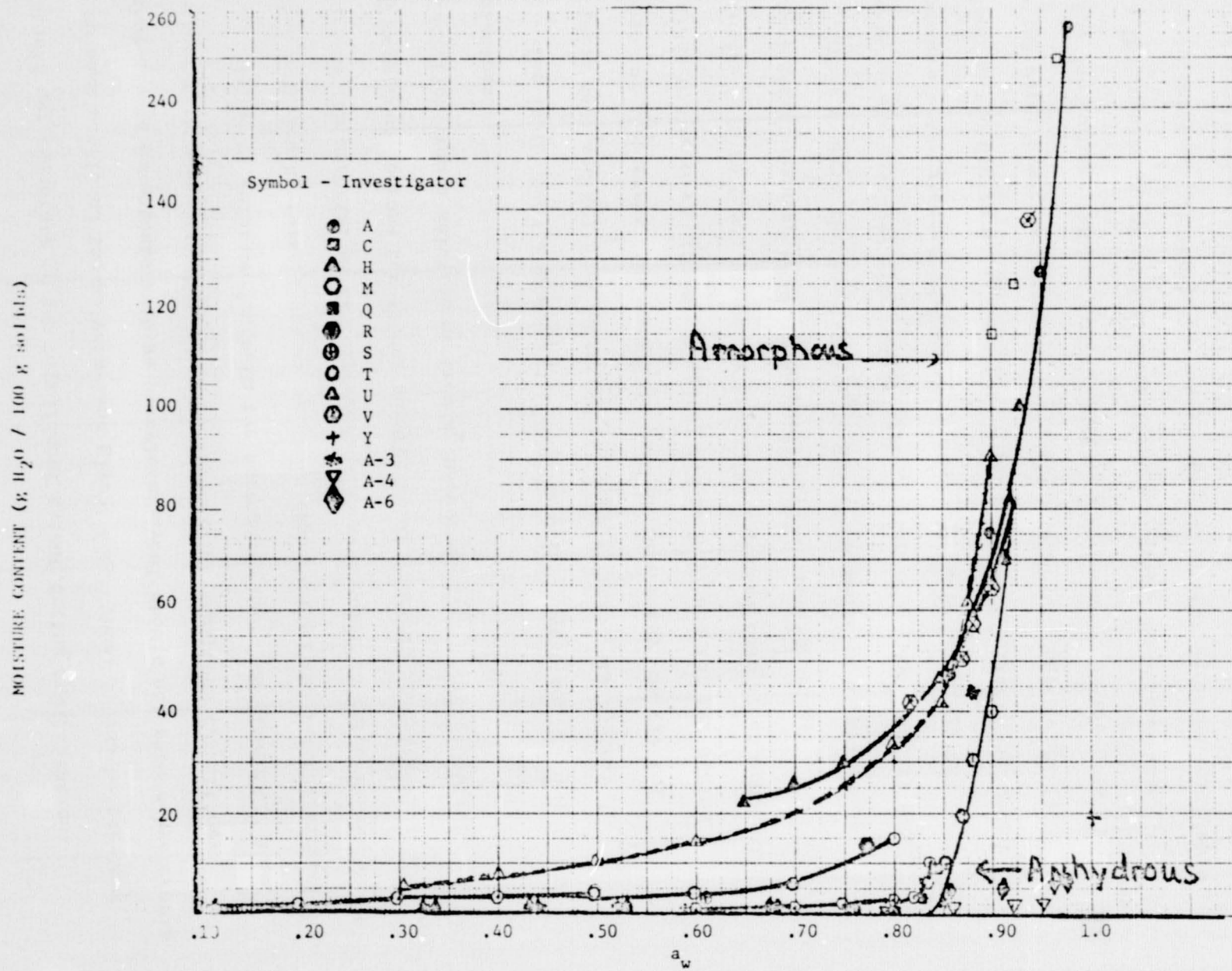
TABLE 4

EFFECT OF ORDER-OF-MIXING OF PROPYLENE GLYCOL IN A

MEAT-SOY FLOUR MODEL SYSTEM

g Propylene glycol added per 50g system	Measured a_w					
	Wet Mix			Dry Mix		
	0 wks	3 wks	12 wks	0 wks	3 wks	12 wks
0	0.89	0.89	0.89	0.89	——	0.89
2	0.89	0.87	0.88	0.88	0.88	0.87
3	0.87	0.87	0.87	——	0.87	0.87
4	0.85	0.85	0.86	0.85	0.85	0.85
5	0.83	0.84	0.84	0.83	——	0.85
7	0.81	0.83	0.83	0.81	0.82	0.82

TABLE 5

EFFECT OF ORDER-OF-MIXING OF SUCROSE

IN A MEAT-SOY FLOUR MODEL SYSTEM

Sucrose g Added/ 50 g system	Sucrose Measured a_w			
	Crystalline sucrose		Amorphous sucrose	
	Wet	Dry	Wet	Dry
0	0.91	0.91	0.91	0.91
4	0.90	0.90	0.89	0.90
8	0.87	0.86	0.87	0.87
12	0.85	0.85	0.85	0.85
16	0.84	0.84	0.84	0.83
20	0.83	0.83	0.83	0.83

the sucrose must be rapidly dissolved in the available water and changed into a solution or amorphous form so that a_w is the same as in the wet mix system. As expected as shown in Table 5 there is also no difference in the order of mixing for the amorphous sucrose. Although the humectant/ water isotherm suggests that amorphous sucrose would lower the a_w of the system to a greater extent than does anhydrous crystalline sucrose, due to its higher degree of hydrogen binding sites, this is not the case in this model food system. There is no significant difference between the a_w lowering ability of crystalline vs. amorphous sucrose as shown in Table 5. This is most likely due to the fact that crystalline sucrose dissolved rapidly as stated before and was changed into a solution or amorphous form.

The results obtained for sodium chloride are shown in Table 6 for two runs. In the second run as indicated not all the salt was able to be predissolved. Although cyrstalline NaCl showed true sorption hysteresis between a_w 0.75 to 0.95, as seen, no true order-of-mixing effect occurred. In addition, the storage conditions did not affect the water activities. In Table 7, no order-of-mixing effect was found for cyrstalline sorbitol. Thus, all crystalline materials dissolved rapidly enough in the pre-added water so that there was no effect or order-of-mixing in the range studied.

In addition to remove storage results Table 8 shows that after 3 weeks of storage time of sucrose at 4°C no significant change in water activity was found.

In conclusion, even though the pure humectant water isotherm may exhibit a hysteresis effect, there is no significant difference in the final a_w achieved as a result of the order-of-mixing, in the a_w range of intermediate moisture food products. As a result of this investigation, it can be concluded that the method of addition of these humectants should make

TABLE 6

EFFECT OF ORDER-OF-MIXING WITH NaCl in MEAT-SOY FLOUR MODEL SYSTEM

g Humectant added per 50 g system	Dry	Wet
Run 1:		
0	0.98	0.98
2	0.94	0.94
4	0.90	0.90
5	0.89	0.89
7	0.86	0.85

	0 wks	3 wks	0 wks	3 wks
Run 2:				
0	0.90	0.89	0.90	0.90
2	0.87	0.87	0.88	0.88
4	0.84	0.84	0.84	0.84
5 partially crystalline	0.81	0.81	0.81	0.80
7 partially crystalline	0.76	0.76	0.76	0.76
8 partially crystalline	0.73	0.72	0.72	0.72

TABLE 7

EFFECT OF ORDER-OF-MIXING OF CRYSTALLINE

SORBITOL IN A MEAT-SOY FLOUR SYSTEM

RUN 1:

g Sorbitol added/50 g system	Measured a_w			
	Wet Mix		Dry Mix	
	0 wks	10 wks	0 wks	10 wks
0	0.90	0.90	0.90	0.89
3	0.90	0.87	0.87	0.87
6	0.88	0.88	0.87	——
8	0.86	0.86	0.86	0.86
10	0.86	0.86	0.85	0.86
12	0.84	0.84	0.83	0.83

RUN 2:

	0 wks	2 wks	0 wks	2 wks
0	0.91	0.91	0.91	0.90
4	0.86	0.86	0.87	0.87
8	0.86	0.86	0.86	0.85
12	0.83	0.82	0.82	0.83
16	0.81	0.80	0.81	0.81
20	0.79	0.79	0.79	0.79

TABLE 8

EFFECT OF ORDER-OF-MIXING WITH EFFECT OF
STORAGE TIME ON A_W FOR CRYSTALLINE SUCROSE

g Sucrose added per 50g system	Wet Mix		Dry Mix	
	0 wks	3 wks	0 time	3 wks
0	0.89	0.90	0.89	0.90
3	0.88	0.89	0.89	0.89
6	0.87	0.88	0.90	0.88
9	0.87	0.86	0.87	0.86
16	0.84	0.84	0.84	0.84

no difference in the resultant a_w value. Also, no significant difference in the a_w lowering ability of amorphous vs. anhydrous sugars was found in the IMF range. This is probably applicable to other materials. It is possible that at a lower a_w a difference may occur, but this would be of little interest for intermediate moisture foods.

5. REFERENCES

Berlin, E., Anderson, B.A. and Pallansch, M.J. 1969. Sorption of water vapor and of nitrogen by genetic variants of α-casein. J. Phys. Chem. 73: 303.

Bettleheim, F.A., Erlich, S.H. 1963. Water vapor sorption of mucopolysaccharides. J. Phys. Chem. 67: 1948.

Bone, D. 1969. Water activity: Its chemistry and applications. Food Product Develop. August, pg. 81.

Bone, D.P. 1973. Water activity in intermediate moisture foods. Food Technol. 27: 71.

Bone, D.P., Ross, K.D., Shannon, E.L., Flanyak, J.R. 1974. Some factors in formulating and processing intermediate moisture foods . Proc. AACC Meeting, Montreal, Canada. October 1974.

Browne, C.A. 1922. Moisture absorptive power of different sugars and carbohydrates under varying conditions of atmospheric humidity. J. Ind. & Eng. Chem. 14: 712.

1,3 Butylene Glycol (S-26-10). Celanese Chemical Company. New York City, New York.

Celanese Chemical Co. 1971. Antimicrobial spectrum of 1,3 butylene glycol. Celanese Chemical Co. New York City, New York.

Cerelose – Dextrose, a highly versatile food ingredient. CPC International Inc. Englewood Cliffs, New Jersey.

Couvillion, J.L. Preservation of foods with 1,3 butylene glycol. Celanese Chemical Co. Technical Report JLC-51-72. April, 1972.

Dittmar, J.H. Hygroscopicity of sugars and sugar mixtures. Ind. Eng. Chem. 27: 333. 1935.

Doherty, C.J. 1972. Soft moist pet foods. Union Carbide Chemicals, New York City, New York.

Fett, H. 1973. Water activity determination in foods in the range 0.80 to 0.99. J. Food Sci. 38: 1097.

Gregg, S.J. and Sing, K.S.W. 1967. Adsorption, surface area and porosity. Academic Press, New York.

Heiss, R. 1967. Haltarbeit und sorptionsverhalten wasserarmen lebensmittel. Springer-Verlag, Berlin.

International Critical Tables. Vol. III. McGraw-Hill, New York. 1926.

Labuza, T.P. 1974a. Theory, Determination and Control of Physical Properties of Food Materials. D. Reidel Publishing Co., Dordrecht, Holland. Chapter 10, pg. 119.

Labuza, T.P., Sloan, A.E., Acott, K., Warmbier, H.C. 1974b. Intermediate moisture foods: Chemical and nutrient stability. Proc. IV Int'l Congress of Food Technol. Madrid, Spain. Sept. 1974.

Labuza, T.P. 1974c. Storage stability and improvement of intermediate moisture foods. NASA Final Report, NAS 9-12560, Phase II, Section III.

Labuza, T.P. 1971. Properties of water as related to the keeping quality of foods. Proc. 3rd Int'l Congress of Food Science and Technology. pg. 618.

Labuza, T.P. 1968. Sorption phenomenon in Foods. Food Technol. 22: 15.

Labuza, T.P., Simon, I. 1969. Surface tension of fruit juices. Food Technol. 23: 96.

Labuza, T.P., Rutnam, M. 1968. Effect of surface active agents on sorption isotherms of a model system. Can. J. Chem. Eng. 46: 364.

Livengood, S.M. 1972. "Humectants – Technology Series Report #38A-T11. Union Carbide Corp., Tarrytown, New York.

Mackenzie, A.P., Luyet, B.J. 1971. Recovery of compressed dehydrated foods. Phase II, U.S. Army Natick Labs Contract DAAG 17-67-C-0126.

Mankower, B., Dye, W.B. 1956. Equilibrium moisture content and crystallization of amorphous sucrose and glucose. Agric. & Food Chem. 4: 72.

Money, R.W., Born, R. 1951. Equilibrium humidity of sugar solutions. J. Sci. Food Agric. 2: 180.

Nelson, T.J. 1949. Hygroscopicity of sugar and other factors affecting retention of quality. Food Technol. 3: 347.

Norrish, R.S. 1966. An equation for the activity coefficients and equilibrium relative humidities of water in confectionary syrups. J. Food Technol. 1: 25.

Parks, G.S., Huffman, H.M., Cattoir, F. 1928. Studies on glass. J. Phys. Chem. 32: 1366.

Patsch, R., Hoehne, C. 1969. The effect of dihydroxy alcohols on the growth of various species of bacteria. Zentral. Bakteriol. TARASIT Insect. Hyg. 211(1): 88-93.

Plitman, M. 1973. Viability of _Staphylococcus_ _aureus_ in intermediate moisture foods. M.S. Thesis. Massachusetts Institute of Technology. Cambridge, Mass.

Rao, K.S. 1941. Hysteresis in sorption. J. Phys. Chem. 45: 500.

Rhodes, H.E. 1975. Overview of United States Pet Food Industry. Cereal Food World. Jan. pg. 5.

Sloan, A.E., Labuza, T.P. 1975a. Humectants - water binding agents for foods. Food Prod. Devel. (in press)

Smith, R.E., Norvell, M.A. 1975. Nutritional Overview of the Pet Food Industry. Cereal Food World. Jan. pg. 8.

Taylor, N.W., Cluskey, J.E., Senti, F.R. 1961. Water sorption of dextrans and wheats at high humidities. J. Phys. Chem. 65: 1810.

U.S. Patent. #3,806,615

Van Arsdel, W.B. 1963. _Food_ _Dehydration_. AVI Publishing Co., Westport, Conn.

White, G.W., Cakebread, S.H. 1966. The glassy state in certain sugar-containing food products. J. Food Technol. 1:73.

Whittier, E.O., Gould, S.P. 1930. Vapor pressures of saturated equilibrated solutions of lactose, sucrose, glucose, galactose. Ind. & Eng. Chem. 22: 77.

Wolf, M., Walker, J.E. and Kapsalis, J.G. Water vapor sorption hysteresis in dehydrated food. J. Agric. Food Chem.

D. Prediction of Water Activity Lowering Ability of Food Humectants

Reprinted on the following pages is a copy of the article

submitted to the <u>Journal of Food Science</u> for publication. The paper

was presented at the 35th Annual Meeting of the Institute of Food

Technologists.

PREDICTION OF WATER ACTIVITY LOWERING

ABILITY OF FOOD HUMECTANTS

A.E. Sloan and T.P. Labuza

Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

1. ABSTRACT

Equations for prediction of the a_w lowering effect of humectants in a complex IMF food system were investigated. Three equations have previously been used to predict a_w lowering effect of simple solutes in solution, but have never been tested in an IMF system. In addition, two equations were derived, a linear slope method and a graphical procedure. Four commonly used food humectants were incorporated into a model IMF system and the resulting a_w's measured. The measured results were compared to the predicted values of these equations. The linear slope method was shown to be the most accurate of the 5 equations studied. It is simple and requires only the initial a_w and moisture content of the system along with a predetermined slope value from the moisture sorption isotherm for each humectant. The Ross derivation also provides a relatively accurate method of predetermining the final a_w in this range.

2. INTRODUCTION

Many hygroscopic chemical compounds are employed by the food industry to bind water in food products. These water binding agents or humectants are incorporated into food systems to lower the water activity (a_w) of the food material in order to increase shelf stability (Labuza et al., 1974b; Karel, 1973).

These humectants are particularly important in the production of intermediate moisture foods (IMF) (Bone et al., 1974). A wide range of humectants including polyols, sugars, and salts are incorporated into these foods to lower the a_w into the intermediate moisture range (Bone, 1973). This is usually between an a_w of 0.65 to 0.90.

When a humectant is incorporated into a food system, it is difficult to predetermine the final a_w of the product, as some humectants lower the a_w of the system to a greater extent than others depending on molecular weight, solubility, and solute interactions (Sloan and Labuza, 1975a; Bone, 1969).

In order to minimize product development time for these products, it would be useful to have a prediction equation for humectant a_w lowering effect in a complex food system containing solids which do not go completely into solution.

3. PREDICTION EQUATIONS AVAILABLE IN THE LITERATURE

Several investigators have derived equations for predicting the a_w lowering effect of simple solutes in solution. The majority of these equations were designed for use by the confectionery industry in order to predict the equilibrium relative humidity (ERH) of sugar solutions, syrups, and confectionery products. The a_w (ERH ÷ 100) of a confectionery product determines whether loss or gain of moisture can occur during storage and whether the foodstuff will be susceptible to microbial

deterioration during storage (Grover, 1947; Mansvelt, 1963; Mansvelt, 1973).
These equations have not been used for most solid IMF foods possibly due to
the non-ideal behavior of the solutes and to food solids/humectant
interactions. Even though correction factors have been established, no one
has published results of the effectiveness of these equations.

The basic equation of a_w prediction, Raoult's Law, is seen in
equation (1).

$$a_w = \gamma \frac{N_1}{N_1 + N_2} = \frac{P}{P_o} \qquad (1)$$

where: γ = activity coefficient

N_1 = moles water in system

N_2 = moles of theoretical solute in system

P = vapor pressure of water above system

P_o = vapor pressure of pure water

Raoult's Law usually cannot be applied due to the deviation in
ideality of humectants beyond a minimum concentration; the inability to
account for food solids/humectant interactions; and the inability to calculate
a molecular weight for undissolved solids.

Hildebrand and Scott (1962) developed equation (2) for the activity
coefficient of binary regular solutions, as a function of solute concentration,
employing Van Laar's approximations on molecular interactions (Toledo, 1973).

$$\ln \gamma_1 = KX_2^2$$

where: γ_1 = activity coefficient of the solvent in the solution

K = a constant

X_2 = mole fraction of the solute

Norrish (1966) of Knecthel Laboratories Ltd. using similar reasoning as that of Hildebrand and Scott (1966) derived equation (3).

$$\ln a_w = \ln X_1 + \ln \gamma$$
$$= \ln X_1 + K_2 X_2^2 \qquad (3)$$

where: a_w = final water activity

X_1 = mol fraction of water

γ = activity coefficient

K_2 = constant for specific humectant = slope of $\ln a_{w/x_1}$ vs. $\ln X_2^2$

X_2 = mole fraction of solute

for predicting the final a_w lowering effect of a single sugar in solution, which he generalized to include multicomponent systems (Equation 4).

$$\ln a_w = \ln X_1 + ((-K_2)^{\frac{1}{2}} X_2 + (-K_3)^{\frac{1}{2}} X_3 + \ldots)^2 \qquad (4)$$

where: a_w = final water activity

K_2, K_3 ... = binary coefficients of components 2 and 3 with water

X_1, X_2, X_3 ... = mole fractions of water components 2 and 3 respectively

This equation does not account for the effect of food solids/humectant interactions, but does account for non-ideality of the solute/water system. Norrish (1966) and Toledo (1973) presented activity coefficient factors for a wide range of solutes which are shown in Table 1.

Grover (1947), on an empirical basis, derived equation (5) for the prediction of water activity of sugar-water solutions.

$$a_w = \frac{ERH}{100} = 104 - 10 (E_s^o) + 0.45 (E_s^o)^2 \qquad (5)$$

where: $E_s^o = E_1 X + E_2 X_2 \ldots E_n X_n \qquad (6)$

E_i = constant for specific component

$X_1 = \dfrac{g \text{ component in system}}{g \text{ } H_2O \text{ in system}}$

This formula predicts the final a_w based on the composition of the system and the weight ratios of each component to the amount of water in the food. Various empirical constants have been determined for several common food

Table 1

Binary Coefficients of Solutes

Norrish Equation

Solute	K Value
Sucrose	-2.70
Corn Syrup DE42	-2.31
Sorbitol	-0.85
Dextrose	-0.70
Fructose	-0.70
NaCl	-7.60
KCl	-4.70
Glycerol	-0.38
Propylene Glycol	-0.20
1,3 Butylene Glycol	-0.20

components and are listed in Table 2. Grover claims this equation to be effective between the a_w range of 0.50 to 0.90 for confectionery items, but it is not considered valid at high concentrations.

Recently, Ross of The Quaker Oats Co. has derived equation (7) based on the Gibbs-Duhem relationship (Ross, 1975).

$$a_f = a_1 \cdot a_{H_1} \cdot a_{H_2} \ldots a_{H_N} \tag{7}$$

where: a_f = final a_w of system + humectant

$\quad a_1$ = initial a_w of food

$\quad\quad$ (no humectant added)

$\quad a_{H_1}$ = a_w of specific humectant/H_2O solution based on total water content

$\quad a_{H_2}$, a_{H_3} = a_w of humectant/H_2O solution based on total water content

$\quad\quad$ for components 2 and 3, respectively

This equation assumed that in a food system, each a_w lowering component behaves independently. The final a_w is a product of each component a_w based on its being dissolved in all of the water in the system. Ross has discussed in detail the problems concerning his prediction equation and presents methods for coping with non-ideality for simple solutions. However, these data have not been applied to most solid food systems.

In the Norrish and Ross prediction equations, a true sorption isotherm is needed to determine the a_w value of a humectant at a given moisture content.

4. MATERIALS AND METHODS

1. **Sorption Experiments.** Sorption experiments were conducted for the humectants listed in Table 3. True adsorption and desorption isotherms were measured for each of these humectants by static moisture gain from dry material using amorphous and/or crystalline material or by moisture loss from a high a_w liquid solution (0.95-0.97). A series of 10 saturated

Table 2

Grover's Food Component

Conversion Factors (E_i)

Sucrose, Lactose	1.0
Invert Sugar	1.3
Gelatin, Casein	1.3
Confectioners Glucose Solids	0.8
Starch	0.8
Gums, Pectin, etc.	0.8
Tartaric and Citric Acids and Their Salts	2.5
Glycerol and Other Glycols	4.0
Sodium Chloride and Other Salts	9.0
Protein	1.3
Corn Syrup DE42	0.8

Table 3

Humectants

Polyols	Supplier
*Propylene Glycol	Dow Chemical
1,3 Butylene Glycol	Celanese Chemical Co.
Glycerol	Mallinckrodt
Polyethylene Glycol 400	Union Carbide Chemicals
*Sorbitol (Crystalline)	ICI, United States

Sugars	
Fructose	J. T. Baker Chemical Co.
*Sucrose	Mallinckrodt
Glucose	J. T. Baker Chemical Co.
Lactose	Eastman Organic Chemicals
Corn Syrup DE42	Clinton Corn Processing Co.

Salts	
KCl	Mallinckrodt
*NaCl	Mallinckrodt

*Humectants used for a_w prediction equation tests.

salt solution desiccators ranging from a_w = 0.11 to 0.97 (Rockland, 1960) were used for periods from 4-8 months at room temperature. In addition, desorption curves were prepared by measuring the a_w of humectant/water solutions by the vapor pressure manometric technique (Labuza, 1974a; Toledo, 1973). The humectant curves and procedures used are discussed in detail by Sloan and Labuza (1975a). The curves may be obtained from the authors.

In addition to our work, literature data on the water sorption properties of these humectants were also collected. Many other methods have been used as well.

2. <u>New Prediction Equations Derived</u>. Due to the complexity of the previous prediction equations, two other prediction equations were derived. The first method, the linear slope method, assumes the food behaves as a humectant. As shown in Figure 1, the food at the initial a_w is assumed to have a theoretical humectant concentration at that a_w from the humectant isotherm. Water activity lowering is then based solely on the increase in weight of humectant above this concentration. The predicted value can then be calculated from the slope of the curve, as shown by equation (8).

$$a_f = a_i - \frac{\gamma \cdot z \cdot (1-W_1)}{W_d}$$
(8)

where: a_f = final a_w

a_i = initial a_w

γ = slope of w/w of solution curve at initial a_w

z = g humectant added

$1-W_1$ = wet basis moisture content

W_d = moisture content of system-wet basis

FIGURE 1

LINEAR SLOPE METHOD

Linear slope $= \gamma \left[\dfrac{a_v}{\alpha/(\alpha + H_1)} \right]$

HUMECTANT/TOTAL WT. (W_T)
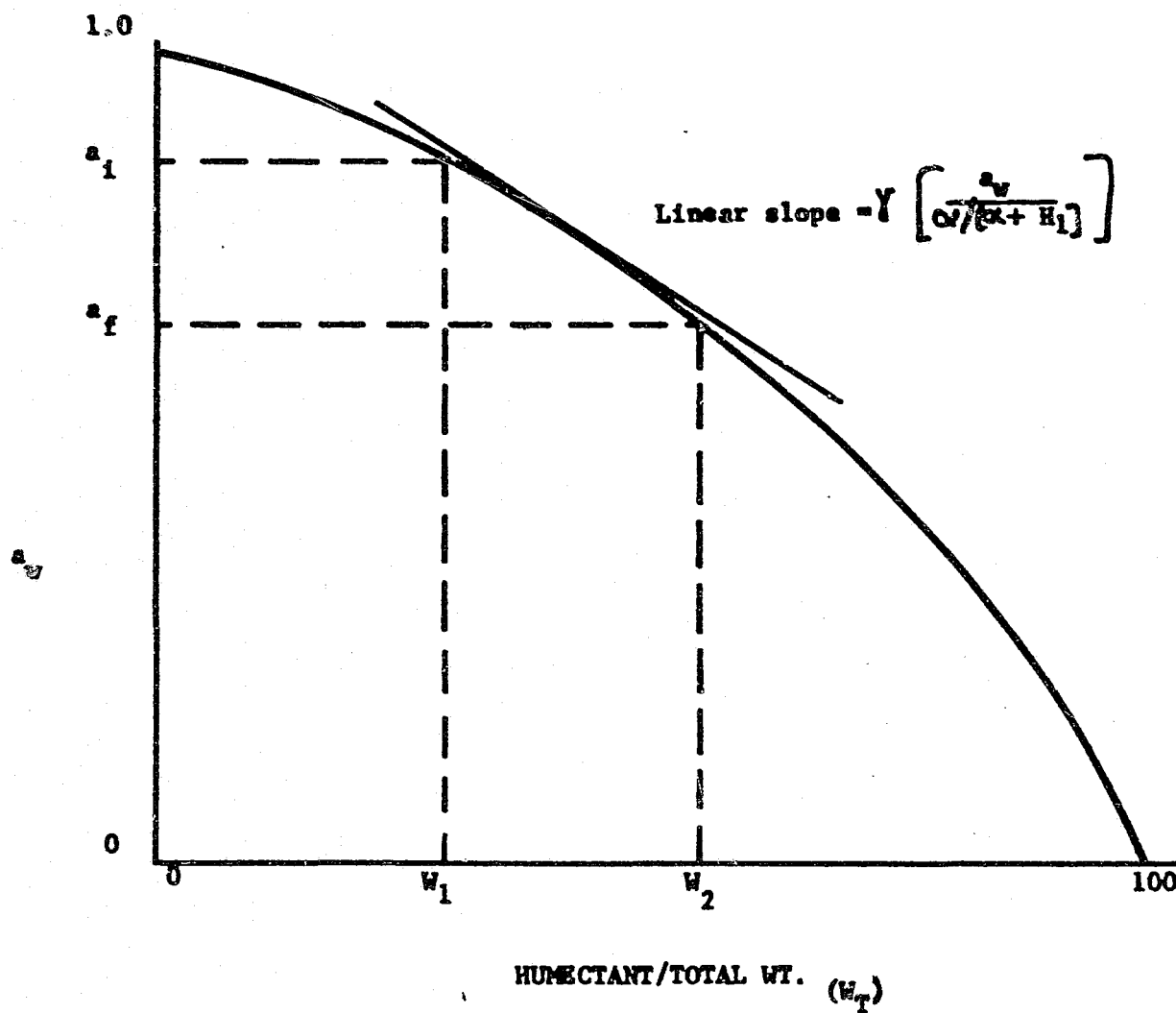
Slope values are shown in Table 4 for all the humectants in the ranges between 0.90 and 0.85. The moisture content at a_w 0.90 is also shown. This method corrects for the initial moisture content of the system. As with the Ross derivation, this method assumes that the food solids behave independently and only contribute to the initial a_w value and moisture content of the food. In this way the equations are similar.

The second method, a simplification of the slope method, is a graphical procedure; however, moisture content correction is not done. With this method, as shown in figure 2, the predicted value can be read directly from a humectant isotherm assuming the food solids to be equivalent to humectant at the same a_w. For this method, only the initial a_w of the food and the humectant isotherm are necessary.

3. <u>Model System for Testing a_w Lowering.</u> A meat-soy flour model intermediate moisture dog food system was employed in this study. The composition of this system is shown in Table 5. Increasing increments of four types of humectants most commonly used in IMF products (Table 3) were each dissolved in a predetermined amount of water, for example, the amount needed to reach an a_w of 0.90 without humectant, and blended for 5 minutes at high speed in a Brabender-Farinograph. The a_w's were measured by the vapor pressure manometric technique (Labuza, 1974a; Toledo, 1973) or the Fett-Vos isopiestic technique (Vos and Labuza, 1974) after 24 hr equilibration at 4°C. The final a_w's were calculated by each of the 5 equations previously discussed.

Table 4

Linear Slope Method

Humectant	Slope	(1-W) at a_w 0.90
Glycerol	0.556	0.68
Propylene Glycol	0.455	0.71
1,3 Butylene Glycol	0.430	0.60
Polyethylene Glycol 400	0.330	0.60
Sorbitol	0.343	0.57
Sucrose	0.182	0.415
Fructose	0.455	0.610
Lactose	0.882	0.030
Glucose	0.360	0.520
Corn Syrup Solids DE42	0.500	0.425
NaCl	0.960	0.975
KCl	0.526	0.825

FIGURE 2

GRAPHICAL PROCEDURE
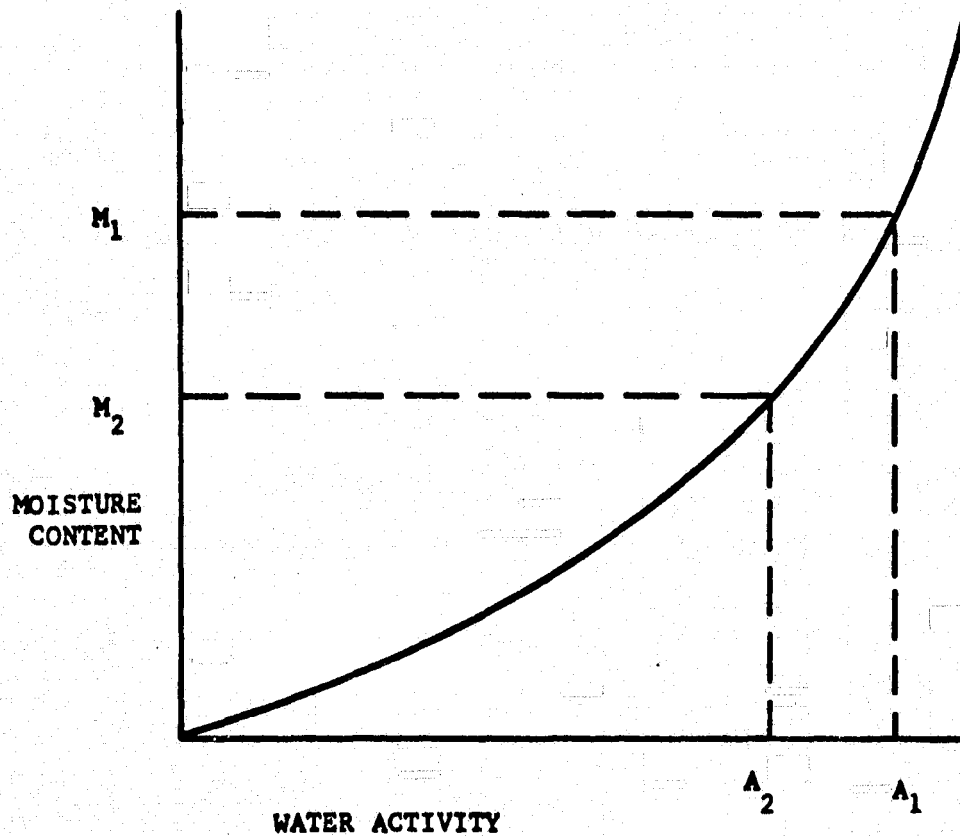
$$\text{AT } A_1 \qquad m_Z = \left[\frac{m_f}{100 + z} \right] \times 100$$

where a_f = final a_w
m_Z = m_c at final a_w
m_1 = initial m_c at a_i
a_1 = initial a_w
z = g humectant added

Table 5

Meat - Soy Flour Model IMF System

	% By Weight
Meat (lean ground hamburger)	19.5
Soy Flour (PDI-20)	78.2
(Cargill, Inc.)	
Citric Acid	2.0
(J. T. Baker Chemical Co.)	
Potassium Sorbate	0.3
(Anheuser-Busch, Inc.)	

5. RESULTS AND DISCUSSION

Figure 3 shows the isotherm measured for sorbitol. As shown, a hysteresis effect exists between the amorphous and anhydrous crystalline forms in which a different moisture content can occur above a given a_w value. Therefore, theoretically, the proper curve for a_w prediction must be used in terms of how the humectant was added to the food. However, as shown by Sloan and Labuza (1975b), even if the humectant is added in a dry state, at least in the high a_w range, the amorphous solution curve can always be used for sugar type material.

Table 6 shows the predicted a_w values and the actual measured values for amorphous sucrose and crystalline sorbitol. As seen, both the Ross method and the linear slope method predict the a_w values quite well, with the linear slope method predicting the a_w values within $\pm$ 0.01 a_w units and the Ross equation predicting final a_w values to within $\pm$ 0.02 units. This is certainly within the error of a_w measurement as described by Labuza et al. (1975). Both the graphical and the Norrish equation consistently predict much higher a_w values than those actually measured. Grover's predictions are also not as accurate as either the linear slope or Ross method.

The results obtained for propylene glycol and NaCl are shown in Table 7. Once again the linear slope method and the Ross derivation most accurately predict the final a_w. The other methods give consistently high a_w values as before.

The prediction variability and the overall a_w deviation obtained for each of the 5 methods employed are shown in Table 8. Both the linear slope method and the Ross equation are the best methods for predicting the final a_w.

FIGURE 3
WATER SORPTION HYSTERESIS SHOWING AMORPHOUS vs CRYSTALLINE MATERIAL FOR SORBITOL
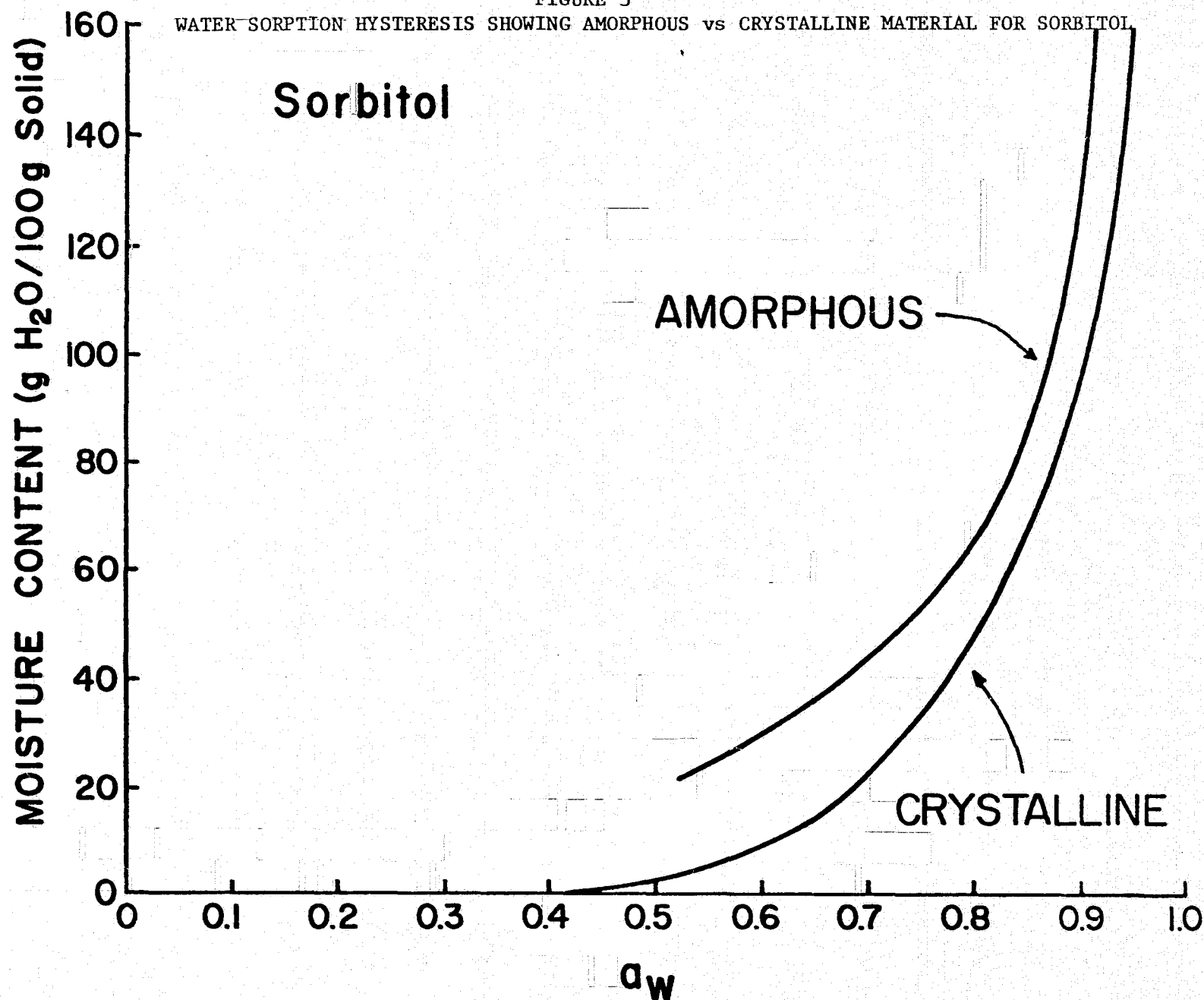
Table 6

Model IMF Dog Food System

Humectant	Measured a_w	Ross	Linear	Graph	Norrish	Grover
Amorphous Sucrose						
g added/100 g system						
4.69	0.94	0.94	0.94	0.95	0.96	0.91
9.38	0.93	0.93	0.93	0.95	0.96	0.90
14.06	0.92	0.92	0.92	0.95	0.96	0.89
20.31	0.91	0.91	0.91	0.94	0.95	0.89
25.00	0.90	0.90	0.89	0.94	0.95	0.88
Crystalline Sorbitol						
g added/100 g system						
4.69	0.88	0.89	0.88	0.89	0.91	0.91
6.25	0.86	0.88	0.87	0.89	0.91	0.90
9.38	0.85	0.87	0.86	0.88	0.91	0.90
14.06	0.84	0.86	0.84	0.88	0.90	0.89
20.31	0.83	0.86	0.84	0.87	0.90	0.89
25.00	0.81	0.80	0.80	0.86	0.88	0.88

Table 7

Model IMF Dog Food System

Humectant	Measured a_w	Ross	Linear	Graph	Norrish	Grover
Propylene Glycol						
g added/100 g system						
3.13	0.88	0.89	0.87	0.89	0.86	0.91
4.69	0.85	0.86	0.86	0.89	0.86	0.89
6.25	0.85	0.85	0.85	0.88	0.85	0.88
7.81	0.83	0.84	0.84	0.88	0.84	0.87
10.94	0.81	0.83	0.82	0.88	0.83	0.85
Sodium Chloride						
g added/100 g system						
2.89	0.94	0.94	0.94	0.98	0.98	0.96
5.71	0.90	0.90	0.89	0.98	0.97	0.90
7.14	0.89	0.87	0.87	0.98	0.97	0.88
10.00	0.85	0.85	0.83	0.98	0.97	0.86

Table 8

Equation Prediction Ability

Method	Overall Average Prediction Variability	Range of Deviation from Predicted Value
Ross	.006	+0.03 to -0.02
Linear Slope	.001	+0.01 to -0.02
Graphical	.044	+0.13 to 0
Norrish	.042	+0.12 to -0.2
Grover	.020	+0.07 to -0.3

Based on these results, the linear slope method has been shown to provide a very accurate means of predicting the final a_w value for a single humectant. It is simple and requires only the initial a_w and moisture content of the system along with a predetermined slope value for each humectant. The Ross derivation also provides a relatively accurate method of predetermining the final a_w in this range. The Grover equation, although not as accurate as the linear slope or Ross methods, is much more accurate than either the graphical or Norrish procedures but requires a long mathematical solution. It is expected that the other humectants for which sorption isotherms were determined will follow the same pattern.

Prediction of the final a_w at higher humectant levels is being investigated. However, at higher levels the humectant would constitute such a major portion of the product that it would be organoleptically unacceptable.

ACKNOWLEDGEMENTS

6. REFERENCES

Bone, D. P. 1969. Water Activity: Its Chemistry and Applications. Food
Product Development. August. p. 81.

Bone, D. P. 1973. Water Activity in Intermediate Moisture Foods.
Food Tech. 27:71.

Bone, D. P., Ross, K. D., Shannon, E. L., and Flanyak, Jr. 1974.
Some Factors in Formulating and Processing Intermediate Moisture Foods.
Proc. AACC Meeting, October, Montreal.

Grover, D. W. 1947. The Keeping Properties of Confectionery as Influenced
by Its Water Vapor Pressure. J. Soc. Chem. Ind. 66:201.

Hildebrand, J. H. and Scott, R. L. 1962. Regular Solutions. Prentice-Hall,
Inc., Englewood Cliffs, N. J.

Karel, M. 1973. Stability of Low and Intermediate Moisture Foods. Proc.
6th Int'l. Course on Freeze Drying and Advanced Food Technology.
Buergenstock-Lucerne, Switzerland. June.

Labuza, T. P. 1974a. Theory, Determination, and Control of Physical
Properties of Food Materials. D. Reidel Pub. Co., Dordrecht, Holland.
Ch. 10, p. 119.

Labuza, T. P., Sloan, A. E., Acott, K. A., and Warmbier, H. C. 1974b.
Intermediate Moisture Foods: Chemical and Nutrient Stability.
Proc. IV Int'l. Congress of Food Tech., Madrid, Spain. September.

Labuza, T. P., Tatini, S. R., Lee, R., Acott, K. A..McCall, W., and
Flink, J. 1975. Determination of Water Activity: A Comparative Study.
J. Fd. Sci. (Submitted).

Mansvelt, J. W. 1963. Effects of Storage on Confectionery. Confectionery
Production. p. 246. November.

Mansvelt, J. W. 1973. Shelf-life of Sugar Confectionery - A Critical

Analysis of Defects in Water Content, Texture, Structure, Chemical

and Microbiological Changes. Confectionery Production. p. 542. Oct.

Norrish, R. S. 1966. An Equation for the Activity Coefficients and

Equilibrium Relative Humidities of Water in Confectionery Syrups.

J. Fd. Tech. 1:25.

Rockland, L. B. 1960. Saturated Salt Solutions for Static Control of

Relative Humidity Between 5 and 40°C. Anal. Chem. 32:1375.

Ross, K. D. 1975. Estimation of Water Activity in Intermediate Moisture

Foods. Fd. Tech. 29:26.

Sloan, A. E. and Labuza, T. P. 1975a. Humectants - Water Binding Agents

for Foods. Food Prod. Dev. (In press).

Sloan, A. E. and Labuza, T. P. 1975b. Effect of Order of Mixing on a_w

Lowering Ability of Food Humectants. J. Fd. Sci. (Submitted).

Toledo, R. T. 1973. Determination of Water Activity in Foods. Proc.

Meat Ind. Res. Conf. AMIF. March.

Vos, P. T. and Labuza, T. P. 1974. Technique for Measurement of Water

Activity in the High a_w Range. J. Ag. Fd. Chem. 22:326.

E.   Water Activity Determination - A Collaborative Study

Reprinted on the following pages is a copy of the article

submitted to the __Journal__ __of__ __Food__ __Science__ for publication.   The paper

was presented at the 35th Annual IFT Meeting.

WATER ACTIVITY DETERMINATION - A COLLABORATIVE STUDY

T.P. Labuza*, K. Acott*, S.R. Tatini*, R.Y. Lee*, J. Flink** and W. McCall***

* Department of Food Science and Nutrition
University of Minnesota

** M.I.T., Department of Nutrition and Food Science
Cambridge, Massachusetts 02139

*** Armour and Company, Oakbrook, Illinois 60521

1. ABSTRACT

Seven methods of water activity (a_w) measurement were tested in a collaborative study between three laboratories. Both saturated salt solutions and foods were measured. When compared to standards, the vapor pressure manometric technique gave the best results. However, the absolute values of the standards are questionable. The results of the comparison of the values for foods showed a range of ± 0.02 a_w units. Thus, one can question the validity of literature values reported to 3 decimal places or the absolute values for limits on microbial growth. This research suggests that some standards must be set for all research groups.

2. INTRODUCTION

Water activity (a_w) is an important property in the manufacture of food systems and formulations. Most chemical reactions and microbiological activity are controled directly by the water activity of the food system as reviewed by Labuza (1974). This study was undertaken because of the lack of a standard method of measuring a_w which would ensure comparable results for different investigators. At present, most investigators have their own method of a_w determination, and in some cases report a_w to three decimal places. It is not known definitely if the method used is that accurate or which method is most precise.

This study has involved the preparation of various saturated salt solutions, commercial foods and model food systems by our laboratory at the University of Minnesota, and the determination of the sample a_w's by us as well as by Dr. J. Flink at Massachusetts Institute of Technology and W. McCall of Armour and Co. Seven techniques or instruments were employed. The methods of a_w determination included in this study were:

1. Brady Array
2. Humichek
3. Relative humidity indicator 400 D
4. Hygrodynamics Hygrometer
5. Sina-scope
6. Equilibrium moisture adsorption (Fett-Vos method)
7. Vapor pressure manometer (VPM)

Saturated salt solutions are usually used for calibration in most methods, so a wide range was used. With but few exceptions, all samples have been analyzed in duplicate by the seven methods of a_w determination. The following points were considered in this study:

1. Did the measured value agree with the theoretical literature

value (in the case of saturated salt solutions)?

2. Did the results of the various methods show good correlation to each other?

3. Was there evidence that volatile glycols, or other properties of the samples, caused interference with the a_w determination?

4. Did any single method seem outstandingly better over the full a_w range for foods or saturated salt slurries?

3. MATERIALS AND METHODS

The saturated salt solutions which were tested in the study were $MgCl_2$, $Mg(NO_3)_2$, $(NH_4)_2SO_4$, $CdCl_2$, Li_2SO_4, Na_2HPO_4, and K_2SO_4. The salts (Analytical Reagent Grade, AR) were obtained from the University of Minnesota Chemical Storehouse. Saturated solutions (slurries) of these salts were made with distilled-deionized water according to the method described in Hygrodynamics Bulletin No. 5 . One large bottle of each slurry served as a source for all of the determinations. However, during the course of the study a question arose as to the purity of the stock slurry of Li_2SO_4 and a small amount of fresh slurry was made to check the results. The two batches had the same a_w as measured by the hygrometer and by the VPM so there was confidence in the purity of the salt.

Aliquots of these samples were decanted into glass 4 oz. jars and covered with parafilm and plastic screw caps. The samples were held at $22^\circ C$ (room temperature) until being sent out for analysis by the various other methods. It has been stressed that the subsamples came from one source because this study involves reproducibility in a_w determination, not in the preparation of a saturated salt slurry per se. It must be assumed that if AR grade salts and "pure" water are used, the a_w of a slurry is consistent for any particular salt.

An exception to the above assumption occurs, however, when the a_w of the slurry is high enough for microbial growth. If an autotrophic bacteria is present, and it can grow on Na^+, H^+ and $PO_4^=$, then the a_w of that slurry may change. In fact, this occurred in a sample of the Na_2HPO_4 slurry in which microbial activity was detected while measuring on the VPM since it showed a continuous increase in pressure due to gas evolution. For this reason, the VPM cannot be used on samples supporting activity microbial activity. The results of other methods of determination would not be affected by this gas evolution from microbial growth.

The foods and model food systems that were analyzed in the study (Table 1) were chosen to represent a wide a_w range; high moisture, intermediate moisture and dry foods. The Thuringer had been fermented, smoked and cooked according to sausage manufacturing conditions. The cheese, bread and intermediate moisture cat food were purchased at a local supermarket. Commercial IM cat food represented a food high in glycols. Hennican, an intermediate moisture model food system, was made according to the composition described by Acott and Labuza (1975). K-sorbate (0.3%) was added to retard spoilage during handling. Two other Hennican systems containing glycols were also tested. The semolina-egg dough was made using Como No. 1 semolina (Capital Duram Division, International Multifoods Corp., Minneapolis) plus 5.5% whole egg solids (A.J. Pietrus and Sons, Co., Sleepy Eye, Minn). Protein hydrolysate powder (PHP, Mead Johnson, Evansville, Ind) and NZ-amine (Sheffield Chemical, Kraftco Corp., Oneonia, New York) systems were made with distilled water as described by Troller (1971). The theoretical a_w of these systems, 0.90 and 0.93, as reported by Troller was based on using a calibrated hygrometer sensor. A commercial dry soup mix was chosen as a typical dry product. The sausage and protein-based bacteriological media

TABLE 1

FOODS ANALYZED IN STUDY

Thuringer sausage

Processed American cheese (Kraft) - individual wrapped
                                                slices

Bread (Wonder Bread) - sandwich type

IM Cat food (Tabby)

Hennican (a_w 0.91)

Hennican plus 2% glycerol

Hennican plus 2% 1,3 butylene glycol and
            2% propylene glycol

Semolina dough mix + 5.5% whole egg solids

Microbiological growth media, NZ-amine and protein
            hydrolysate, 0.90 (Troller, 1971)

Microbiological growth media, NZ-amine and protein
            hydrolysate, 0.93 (Troller, 1971)

Dry soup mix (Lipton vegetable soup)

were prepared by Mr. Robert Lee and Dr. Sita R. Tatini of the Department of Food Science and Nutrition, University of Minnesota.

Preparation and Handling of Samples:

The saturated salt slurries were stored in 4 oz. jars covered by parafilm and a plastic screw cap. The salts were held at room temperature and equilibrated at the temperature of analysis for 24 hr prior to analysis. It is important to note that these were salt slurries. They consisted of a large excess of crystalline salt in a saturated solution which was just sufficient in volume to cover the salt crystals.

In general, room temperature storage of salt slurries is sufficient. Those of $a_w \geq 0.97$ should be stored at 4°C to prevent microbial activity or made up fresh and held at room temperature for only a limited amount of time. If room temperature storage were preferred, the possibility of contamination by microbes could be reduced by using sterile water and glassware, and aseptic techniques. Slurries of $a_w \geq 0.97$ made and stored in this way should still be examined closely for microbial activity as the salt crystals themselves may carry some microbial contaminants.

The foods and model food systems were packed in 202 x 214 epoxy-lined tin cans. The loaves of the commercial white bread, packages of the individually wrapped slices of American processed cheese, the soup mix and the cat food were purchased on the day prior to canning. Nine slices of bread were stacked up and the can was used to stamp out the centers of the slices. No crust was used. The compacted bread was then sealed in the cans. The cheese was unwrapped, cut into 1 cm strips and tightly stacked into the cans, leaving little air space. The cat food was ground in a blender to ensure a homogeneous sample. The soup mix was tested as it came from the manufacturer's pouch. The cans were sealed immediately.

The Hennican systems and semolina-egg dough was also packed into cans and sealed. In all cases the food was at room temperature at the time of canning and sealing.

Precautions were taken to keep microbial contamination to a minimum. The cans were sanitized with 500 ppm hypochorite solution. Sterile gloves were worn during handling of the cheese and bread. Sterile wooden blades were used to transfer the Hennican.

The bacteriological media, NZ-amine - PHP, was made in 2 batches, one at each a_w. After mixing and solubilizing all the protein the batches were poured into four 250 ml Nalgene bottles with screw caps. The bottles were filled half-full to leave space for the foaming which occurred during autoclaving. The samples were autoclaved for 15 min and then brought to atmospheric pressure very slowly. The sterile media was cooled to room temperature; then the caps were tightened.

Instructions on sample handling were sent to investigators prior to sending the samples. The salts were held at room temperature, the perishable foods were kept at 4°C (except for the 24 hr pre-analysis temperature equilibration), and the sterile media were kept at room temperature and not transferred or opened until just prior to analysis. Samples were prepared and sent on two separate occasions to the other investigators. In some cases due to instrument problems, not all samples were tested by each method as indicated in the results by "not tested".

The analyses were performed at 21-23°C. The food samples were transferred into 4 oz. snap cap vials 24 hr prior to analysis. If a hygrometer was used the sensor mounted in a cover for the vial was fixed over the sample, replacing the original cover and forming an air-tight seal. The procedure from this point was performed as necessary for each instrument.

The values for the standards (saturated salt solutions) used to calibrate the instruments were established individually by each investigator taking an average of values found in the literature. The standard salt solutions and references used by M.I.T. appear in Table 2 and those used by the University of Minnesota in Table 3. The measurements at Armour were only made on the Sina-scope instrument and the instrument was calibrated as will be described later. Table 4 lists a comparison of the average values for the salts from Tables 2 and 3. The average value from the M.I.T. data was obtained by averaging the 20° and $25^\circ C$ data from references 1 through 8 of Table 2 while only the 20° data from Table 3 were used for comparison. The standard values were the same in all cases except for three salts, $KC_2H_3O_2$, $Ca(NO_3)_2$, and KNO_3 where a 0.01-0.02 deviation was seen. Since most values are the same, the results from M.I.T. should be directly comparable with the University of Minnesota results, however, this shows the problem in relying on literature values and averages in preparing calibrations.

The Brady Array (Thunder Scientific Co., Albequerque, New Mexico) is a bulk-effect device. The manufacturer suggests that this mode of sensor equilibration is much faster than the surface type devices. The interaction occurs within the structure of the sensor. It consists of a precise array of crystal semiconductor junctions and spaces. The presence of water molecules imposes stress on the bonds of the crystal lattice structure. As the stress increases, the bonds become distorted. Energy is released to the free electrons within the structure effecting an increase in conductivity. The change in conductivity is used as a measure of the concentration of water molecules in the lattice, and this is related via an electrical signal to % relative humidity (Bennewitz, 1973).

TABLE 2

WATER ACTIVITY VALUES FROM THE LITERATURE (M.I.T.)

Salt	(1) 22°C	(2) 20°C	(3) 22°C	(4) 20°C	(5) 25°C	(6) 25°C	(7) 20°C	(8) ~20°C	(9) 20°C
$KC_2H_3O_2$	0.230	0.23	0.215	0.200	0.225*	0.227*	---	---	0.20
$MgCl_2$	0.33	0.33	0.325	0.340 / 0.331*	0.330*	0.324*	0.336 / 0.332*	---	0.33
$Zn(NO_3)_2$	---	0.38	---	0.420	---	---	---	---	0.42
$Ca(NO_3)_2$	0.522	0.56	0.507	0.560 / 0.558*	---	---	---	---	---
$Na_2Cr_2O_7$	0.582	---	---	0.520	---	0.536*	0.552 / 0.538*	0.542*	0.52
$NaNO_2$	0.648	---	---	0.660	---	---	---	0.630**	0.66
$NaCl$	0.756	0.75	---	0.765 / 0.758*	0.753*	---	0.755	0.757	0.76 / 0.75**
$(NH_4)_2SO_4$	0.802	0.79	---	0.817 / 0.810*	---	---	0.806	---	0.81 / 0.81**
KNO_3	---	0.94	0.920	0.942 / 0.930*	0.925*	---	0.932 / 0.920*	---	0.94 / 0.93**

(1) Wink and Sears, TAPPI (1950)
(2) Rockland (1960)
(3) Thunder data sheet on Calibration Cells
(4) O'Brien, 25: 73 (1948)

(5) Stokes and Robinson (1949)
(6) Richardson and Malthus (1955)
(7) Wexler and Hasegawa (1954)
(8) Carr and Harris (1949)
(9) International Critical Tables (1926)

*   25°C
** 30°C

TABLE 3

WATER ACTIVITY VALUES FOR SATURATED SALT SOLUTIONS FROM THE LITERATURE
U of MN

Saturated salt solutions	°C	Int'l Crit. Tables 1926	Wexler & Hasegawa 1954	Rockland 1960	Handbook of Chem. Phys. 1972-73	Average Value
$LiCl \cdot H_2O$	20	0.15	0.124	0.12	0.15	0.14
	25	--	0.120	0.11	--	0.12
$KC_2H_3O_2$	20	0.20	---	0.23	0.20	0.21
	25	--	---	0.23	--	0.23
$MgCl_2 \cdot 6H_2O$	20	0.33	0.336	0.33	--	0.33
	25	0.32	0.332	0.33	--	0.33
KCO_3	20	--	---	0.44	0.44	0.44
	25	--	---	0.43	0.43	0.43
$Mg(NO_3)_2 \cdot 6H_2O$	20	0.55	0.549	0.52	--	0.54
	25	0.52	0.534	0.52	0.52	0.52
$NaCl$	20	0.76	0.755	0.75	--	0.76
	25	0.75	0.758	0.75	--	0.75
$(NH_4)_2SO_4$	20	0.81	0.806	0.79	0.81	0.81
	25	0.81	0.803	0.79	0.81	0.80
$CdCl_2$	20	--	---	0.82	--	0.82
	25	--	---	0.82	--	0.82
Li_2SO_4	20	--	---	0.85	--	0.85
	25	--	---	0.85	--	0.85
K_2CrO_4	20	0.88	---	0.88	0.88	0.88
	25	--	---	0.87	--	0.87
KNO_3	20	0.94	0.932	0.94	--	0.94
	25	0.93	0.920	0.93	--	0.93
Na_2HPO_4	20	0.95	---	0.98	0.95	0.96
	25	--	---	0.97	--	0.97
K_2SO_4	20	0.97	0.972	0.97	--	0.97
	25	0.97	0.969	0.97	--	0.97

TABLE 4

STANDARD A_W VALUES DETERMINED BY AVERAGING

Saturated salt solutions	M.I.T. Standard Value *	Univ. of Minnesota Standard Value **
$KC_2H_3O_2$	0.23	0.22
$MgCl_2$	0.33	0.33
$Zn(NO_3)_2$	0.40	0.40
$Ca(NO_3)_2$	0.54	0.56
$NaCr_2O_7$	0.54	0.54
$NaNO_2$	0.66	0.66
$NaCl$	0.76	0.76
$(NH_4)_2SO_4$	0.81	0.81
KNO_3	0.93	0.94

* Average of data from references 1 through 8 in Tables 2, 20 and 25°C data

** Average of data from references 2, 7 and 9 in Table 3, 20°C data

The Brady Array system as used by M.I.T. in this study consisted of a BR-101R Brady Array connected by cable (C-3A) to a signal conditioning module (SC-1020M). Voltage output was read on a digital output device. A strip chart recorder (Heath IR-18M) simultaneously produced a graph which showed when vapor space equilibrium had been reached. Although this system has been claimed to give low hysteresis effects (Bennewitz, 1973), severe hysteresis was experienced. Furthermore, there is no direct correlation of voltage output with known a_w values. Because of this, constant standardization of the instrument was necessary.

The procedure for the Brady Array was as follows:

1. Determine the recorder reading which corresponds to the theoretical a_w of a primary standard salt solution. All measurements were in the adsorption direction, i.e. the salt of theoretical a_w below that expected for the driest sample, was tested first. The sensor was sealed over the sample. The recorder results showed that the a_w values after 20 or 30 min had not changed significantly, therefore, the 30 min reading was used. The theoretical a_w of the standard salt solution was taken from the M.I.T. standard value in Table 2. The recorder value for the standard was plotted on a graph vs. a_w.

2. Determine the recorder reading that results from 30 min exposure of the sensor to the unknown sample. No desiccation of the sensor was done between these samples.

3. Determine the 30 min recorder reading that results from a standard salt solution of an a_w near, but above, that expected for the unknown. Plot this value vs. a_w and draw a line connecting the values for the two known salts. Interpolate the recorder reading of the unknown and read off the a_w on this line.

4. Determinations of samples of higher a_w were done then, continuing in the adsorption mode and interrupting every 1 or 2 samples by a standard salt solution.

5. When samples of lower a_w were subsequently analyzed, the sensor was held over drierite until the recorder value showed desorption to a_w 0.0, which took about ½ to 1 hr.

6. When not in use, the sensor was stored over drierite.

A plot of the recorder output vs. a_w values of standard salt slurries was not reproducible from day to day. Thus, the interpolation method described above was found to be necessary for every determination. The range of best sensitivity was found to be from 0.4-0.8 a_w because of a sigmoid curve that results between a_w and voltage. The manufacturers claim that signal conditioning can create a curve that is linear from 0-1.0 a_w. The National Bureau of Standards has published a report (#NBSIR-74-477) on the Brady Array which mentions problems including those found in this study.

The Humichek (Beckman Instruments, Cedar Grove, NJ) is an electric hygrometer and was tested at M.I.T. The sensors for the instrument used in this study were modified so that they could be used with the 4 oz. snap cap vials. The sensor was remounted in a 9-pin miniature tube socket into a #7 rubber stopper. Although very little hysteresis was experienced, samples were always tested in an adsorption mode.

The procedure for the Humichek was as follows:

1. The sensor was inserted into the vial forming an air-tight seal.

2. The instrument thumbwheel was adjusted to balance the bridge signal. Two LED lamps indicated high and low reading. The thumbwheel was calibrated so that a direct reading of % relative humidity (RH) (a_w x 100) may be taken

over a range of 15-95% RH (0.15 - 0.95 a_w).

3. a_w values were checked until successive readings made 5 min apart gave a change of less than 0.005 a_w. For salt solutions, this took about 30 min; for the foods about 1 hr.

4. When not in use, the sensor was stored at ambient a_w (approximately 0.25) or over saturated $MgCl_2$ solution.

The thumbwheel calibration was checked against standard saturated salt slurries and was found to be linear between a_w 0.35 and 0.93.

The Relative Humidity Indicator, Model 400 D (General Eastern Corp., Watertown, Mass) is another type of electric hygrometer and was tested at M.I.T. The sensor is sensitive to changes in resistivity of a sulfonated polystyrene matrix as a function of the water molecules present (General Eastern Corp., Bulletin #400-0174). This model gives a digital readout of % RH. For this study, a proportional signal was recorded simultaneously by a Heath IR-18M strip chart recorder. Since the instrument was not correctly calibrated as it came from the manufacturer, a new standard curve was generated using saturated salt solutions and was used to correct the readings. Determinations were done in the adsorption mode.

The procedure for the humidity indicator was as follows:

1. The sensor was sealed over the sample in the 4 oz. snap cap vial.

2. A reading was taken from the digital display when the recorder indicated equilibration of the sensor in the air space over the sample.

3. The reading was corrected by using the calibration curve to obtain the experimentally determined a_w.

The hygrometer (Hygrodynamics, Silver Springs, MD) study done at the Univ. Minn. required the use of several sensors since they are accurate only over particular a_w ranges for which the manufacturer supplies a graph

of reading value vs. % relative humidities. Since the sensors used were of varied and uncertain age and history, recalibration of each sensor was necessary so that a correction factor could be applied to the graph. The correction factor should be the same regardless of the salt used in calibration as long as it was within that sensor's range of sensitivity. This correction factor should also be applicable and accurate over the full range.

Most investigators have used variable periods of equilibration time before reading time such as 20-30 min (Fett, 1973); 8 hr (Plitman et al. 1973); and 24 hr (Vos and Labuza, 1974). The uncertainty in length of time stems from the fact that Hygrodynamics does not give a recommended time for analysis. The manual does state that a sensor will reach 65% of the equilibrium value in 3 min (Bulletin #SB-20). A time course on the equilibration of two of the sensors used in this study showed that after 24 hr the sensor had reached a maximum value, thus 24 hr was used for all samples which should be more than adequate for all sensors or sample types.

The procedure for the Hygrometer was as follows:

1. Samples with a wide range in expected a_w were chosen for one day's analysis. The samples were put into 4 oz. screw-cap jars to half-full, but not touching the probe which was in the jar lid. The appropriate sensors were sealed onto the jars. Parafilm was stretched around the sensor end of the jar to ensure a moisture barrier.

2. After 24 hr $\pm$ 0.5 hr, the sensor was connected to the instrument and a reading was taken from the Hygrodynamics meter.

3. The sensor was then removed and placed in a vacuum desiccator over drierite and a vacuum was pulled. The desorption process got rid of

water as well as other volatiles like glycerol and propylene glycol, which could adsorb on it.

4. After 24 hr the sensor was taken from the desiccator and sealed over the next sample to be analyzed.

5. All determinations, samples and standards were done in duplicate. After a correction factor was determined for a sensor, it was used in analysis of unknown samples.

6. When all samples for a particular sensor had been analyzed (including the 24 hr desorption process), each sensor was recalibrated over the same saturated salt slurry that was used to establish the correction factor. This was done by sealing the "dry" sensor over the slurry for 24 hr and then taking a reading as described above.

It was found that some sensors gave different correction factors for different salts. The results are shown in Table 5. The sensors for the lower a_w range gave reproducible correction factors. The red, yellow and green sensors maintained the same correction factor throughout the course of the study. These sensors were not tested against a second salt. Most sensors for the higher a_w range changed their correction factor during the course of the study from 0.01 to 0.11 a_w units. In addition, one of the violet sensors (#433243) required a different correction factor depending on the saturated salt slurry used for calibration. With NaCl the factor was +0.05 to +0.06, but with the Li_2SO_4 it was +0.12. This is unexpected and undesirable and suggests that the calibration curves and correction factors are not linear. When this problem was discovered, the calibration factor applied was the one established nearest to the time of analysis of each particular sample. This could not be based on a value near the a_w of the sample, since that was unknown until after a correction factor was applied.

TABLE 5

CHANGE IN CALIBRATION WITH TIME FOR THE HYGRODYNAMICS HYGROMETER

Sensor	Sensor number	Calibration salt	Theoretical a_w of salt slurry*	Correction factor for sensor	Check on correction factor	Difference
Red	405481	$KC_2H_3O_2$	0.21	+0.08	+0.08	0
Yellow	433030	KCO_3	0.44	+0.04	+0.04	0
Green	429628	KCO_3	0.44	+0.02	+0.02	0
Violet	433243	NaCl	0.76	+0.06	+0.05	0.01
		Li_2SO_4	0.85	+0.12	+0.12	0
Violet	606052	Li_2SO_4	0.85	+0.02	−0.03	0.05
		NaCl	0.76	+0.02	−0.05	0.07
Gray	541550	KNO_3	0.94	−0.02	−0.01	0.01
Gray	613713	KNO_3	0.94	+0.09	+0.07	0.02
Gray	613741	KNO_3	0.94	+0.07	+0.03	0.04
Gray	613732	K_2SO_4	0.97	+0.12	+0.01	0.11

* Average values determined from references shown in Table 3

The samples to be tested by the Sina-scope (Sina Ltd., Zurich, Switzerland, marketed in the U.S. by Beckman) were performed by W. McCall at Armour Food Co., Oakbrook, Illinois. The a_w was determined on a Sina-scope equipped with an indicator/recorder which indicated when equilibrium had occurred between food, air space and the sensor element. Since the sensing system and sample space are in close contact, equilibration is rapid and occurred within 30 min to 1 hr. The system calibration was expanded to measure a_w over the range of 0.76 to 1.0. Other ranges are available as well as one tocover from 0.5 to 1.0. For this study the dry soup mix was out of the range used thereby making accurate reading impossible. The Sina-scope sensor was equipped with a mechanical filter to protect it from dust, oil and water vapor condensation. A chemical filter can be used to protect the sensor from chlorine, formaldehyde, ammonia, sulfur dioxide, hydrogen sulphide, amino-acids, hydrocarbons and oil droplets. Thus, volatile chemicals in the samples should not cause interference from glycols in the cat food or Hennican. This is a definite advantage over the Hygrometer sensors. This filter was unavailable during this study, however, the necessity for such a filter may be realized by the results found.

The procedure for the Sina-scope was as follows:

1. The sample was put into a plastic sina dish after room temperature equilibration. The open dish was put into the base and the Sina-scope sensor was screwed down forming an air-tight seal.

2. The manufacturer furnishes check tablets which are combined with a few drops of water to check the calibration of the instrument. The tablets are salts and thus saturated solutions are supposedly formed. However, using their procedure, it was obvious that too much dry salt was exposed in the air space which can result in an incorrect calibration. In spite of this, the manufacturer's calibration procedure was used.

3. The indicator/recorder was switched on and time was allowed for the recorder chart to indicate equilibration. The % relative humidity (RH) is then read directly from a bar scale and corrected with the calibration.

The handbook for the Sina-scope references Wexler and Hasegawa (1954) for the a_w of the salts they suggest for calibrating the sensor. Unfortunately, mistakes were made in referencing this work. They quote the values to three decimal places despite the author's qualification of the values for salts. Wexler and Hasegawa used the dewpoint measurement to determine the a_w resulting from various salts. They then plotted data for the salts from 20 different references on the graphs of the curves they obtained. It showed the scattering of data for a salt by different authors using different techniques; the standard deviation in most cases being ±0.03 a_w units and with one salt, magnesium nitrate, ±0.06 a_w. Thus, again an average value may not be the true value.

The isopeistic method of a_w determination was modified from that described by Fett (1973) in which sodium caseinate was the adsorption substrate. The procedure described by Vos and Labuza (1974) was followed using microcrystalline cellulose as the adsorption substrate. The cellulose is a more stable and thus superior substrate. This hybridized method is called the Fett-Vos isopeistic method of a_w determination and was done at the University of Minnesota.

The procedure for the isopeistic or equilibrium moisture adsorption method was as follows:

1. Approximately 100g of the sample was placed in the bottom of a 215 cm plastic desiccator (vacuum type).

2. Duplicate samples of 1.6g (to 0.0001g) of predried microcrystalline cellulose (Avicel, PH-101) were weighed into 35ml glass weighing dishes

with lids. The dishes (without lids) were then placed on the porcelain plates in the desiccators over the food.

3. The desiccators were closed, evacuated for about 1 min and then held at 22°C for 24 hr.

4. After 24 hr, the vacuum on the desiccators was very slowly released. Rapid air current within the desiccator will result in a loss of cellulose from the weighing dishes and failure of that determination. If the desiccator has not maintained a good vacuum during the 24 hr isopeistic procedure, the data from the sample within will be erroneous.

5. The dish (with the lid replaced) and the cellulose were reweighed and the change in weight recorded. The moisture content (g H_2O/g dry cellulose) was calculated and the a_w read off the standard cellulose curve which was prepared previously.

This method of a_w determination is not recommended for samples that are subject to foaming, such as protein solutions. The protein samples (HPH and NZ-amine and H_2O) foamed excessively during the evacuation of the desiccators. A slight modification in procedure was used for these two samples. The samples were degased prior to evacuation. The degasing was done by putting the sample in a desiccator and evacuating for 3 min interrupting periodically to prevent sucking the sample out through the vacuum hose. When degased the sample boiled but the profuse foaming was eliminated. During the procedure 0.5g of water vapor/100g sample was lost ; probably not enough to alter the a_w.

Microbial growth during the 24 hr equilibration must be prevented. This is possible by the addition of antimicrobial agents (K-sorbate) and by using aseptic technique.

The range of accuracy of this method is limited by the shape of the cellulose isotherm. The best accuracy is found in the high a_w range, between a_w 0.81 and 0.96, the IMF range and slightly above. This procedure is highly applicable for heterogeneous samples due to the large sample size used. Application of the Fett-Vos method for samples containing volatiles such as propylene glycol or 1,3 butylene glycol should be exercised cautiously. Adsorption of vapors other than water vapor onto the cellulose will provide erroneous results.

The vapor pressure manometric method (VPM) is one of the best methods for a_w determination as it gives a direct measure of the vapor pressure exerted by the sample. Devices based on this method have been described by Taylor (1961) and Labuza (1974). The a_w is calculated from the ratio of the vapor pressure of the sample to that of pure water at the same temperature. This method was performed at the Univ. of Minnesota.

The procedure for the VPM method was as follows:

1. A 10-30g sample was put into a special 50g sample flask (with 24/40 top) and sealed onto the apparatus.

2. The airspace in the apparatus is evacuated (via vacuum pump) to less than 200 microns (sample is excluded).

3. The space in the sample flask was then evacuated for 30 sec to 2 min depending on the sample. This time should be kept to a minimum to prevent loss of H_2O from saturated solutions however this is not of great significance when following the above procedure as long as it does not actually dry out. Then the stopcock across the manometer is closed.

4. The level of oil in the manometer (Apeizon B oil) will respond to the vapor pressure exerted by the sample. The system was equilibrated at a constant temperature until the manometer oil showed no change in

height. This usually took 40-60 min.

5. The difference in height of the legs of the manometer was recorded as ΔH_1 at the temperature of equilibration. The sample should be water jacketed. Two thermometers, one in the water jacket and one in the air hung from the VPM frame, were used for temperature equilibration. Temperature equilibrium exists when these two precalibrated thermometers showed the same temperature for at least 10 min.

6. The stopcock over the sample was closed and the one over the desiccant was opened. The moisture in the air space of the system absorbs onto the desiccant. As this occurs, the manometer oil lowers. After no further change in the manometer leg occurs, usually 10-20 min, the difference in the legs was recorded as ΔH_2. This difference was due to gases and volatiles lost from the sample and air that leaked into the system.

7. The pressure exerted by the sample water vapor, $\Delta H_1 - \Delta H_2$ (cm), was divided by the pressure that pure H_2O would exert at the same temperature to give the water activity.

8. The drierite stopcock was then opened and the drierite and flask are evacuated. The vacuum was broken on the sample by passing air into the flask through a 3-way stopcock. The sample is taken down from the apparatus.

9. The airspace throughout the system was again evacuated for 15 min. It was then ready for the next sample.

Temperature control is one of the most critical factors involved in accurate determination of a_w by the VPM. Sample and air measurement by thermocouples connected to a multipoint recorder should be used to ensure equivalence of temperature. Volatiles other than water may contribute to the pressure exerted by the food, giving erroneous results. Considerable

work on this question concerning humectants like glycerol and propylene glycol has shown that these glycols have no significant effect on the a_w determination.

It is impossible to perform a_w determination on samples containing high numbers of bacteria or mold. Their respiration prevents vapor pressure equilibrium. The oil in the manometer changes constantly with time. This subject has been discussed previously with regard to saturated salt solutions. The same problem was experienced with a sample of uncooked Thuringer. After cooking, the bacteria were inactivated and a reasonable a_w was obtained. Another disadvantage (or uncertainty) of a_w determinations by the VPM is that some moisture is lost during the evacuation step of the procedure. This could be critical due to the small sample size. The actual loss for each type of sample may vary depending on the functional properties of the sample, e.g. texture, viscosity and porosity. Sood and Heldman (1974) have shown this to be insignificant for foods of low moisture. In one of our tests, a sample of 30g of high moisture cheese (40%) was evacuated for ½ min, 1 min and 2 min and weighed after each time period.

The results showed that in 30 sec less than 0.05g of water was lost from 30g of cheese, while in two min only 0.14g was lost. Thus, about 1% of the water was lost from the cheese. Since cheese is at a high a_w with a steep isotherm, the effect on a_w should be very small. From this is was felt that the normal evacuation of a sample (30 sec to 1 min) will not significantly alter the a_w of high a_w samples since most isotherms are steep above 0.80.

A test of measurement precision was performed using the devices at the University of Minnesota. Five determinations for Li_2SO_4 were done by the Hygrometer, the Fett-Vos and the VPM methods. The temperature during the analysis was 22°C ± 1°C. The Li_2SO_4 was made up in one batch

of slurry by adding distilled-deionized water as usual. All glass

utensils were used in its preparation. Dirt and metal ions can affect

the vapor pressure of salt slurries. Samples of this slurry were taken

for analysis by the three methods. The procedure for each method has

been discussed before.

4. RESULTS AND DISCUSSION

As seen in Table 6, the VPM gave the best precision in the limited

study on Li_2SO_4. However, the VPM value was low when compared to the

standard values listed in Table 3. The Fett-Vos procedure also gave

excellent precision, but readings high by 0.03 units. The Hygrometer

readings were low, and had a standard deviation of ±0.04. The values

for Li_2SO_4 ranged from 0.80 to 0.91. Based on these results, one should

not rely on the standard value reported but one could use the VPM or

Fett-Vos method with a correction factor for an accepted value. Why

the values differ from the literature is not known, however, it is

believed that the literature values may not be correct.

The results of the collaborative study are presented in Table 7

for the values obtained for the standard saturated salt slurries and

in Table 8 for the foods and food systems. The average value for each

duplicate determination is listed. It is suggested that an average of

duplicate determinations always be made. As seen in Table 7, the VPM

gave results that were closest to the theoretical values listed in Table 4.

The average difference from theoretical was 0.01 a_w unit. The electric

hygrometers, Humidity Indicator 400D, Humichek, Hygrometer and the

Sina-scope all had an average deviation from theoretical of 0.02. The

range of the particular Sina-scope used was limited to a_w >0.81 and the

Humichek at a_w >0.80. The Brady Array had the largest deviation from the

TABLE 6

TEST OF PRECISION ON Li_2SO_4 at $22^{\circ}C$

Trial	VPM - $22^{\circ}C$ a_w value		Hygrometer - $22^{\circ}C$ a_w value		Fett-Vos - $22^{\circ}C$ a_w value	
1	0.82		0.88		0.88	
2	0.82		0.83		0.88	
3	0.82		0.81		0.88	
4	0.82		0.78		0.88	
5	0.83		0.85		0.88	
Absolute Average	0.83	±0.01	0.83	±0.03	0.88	±0
1*	0.83		0.87		0.87	
2*	0.83		0.84		0.87	
3*	0.84		0.91		0.85	
4*	0.83		0.80			
5*			0.80			
Overall Average	0.83	±0.01	0.84	±0.04	0.87	±0.01

Average of all results = 0.845

Rockland value = 0.85

* Previous studies over two year period in lab

TABLE 7

A_W RESULTS FOR SATURATED SALT SLURRIES

| Material | Theoretical a_w (Table 4) | Direct VPM | Electric Hygrosensors | | | Sina-scope | Resonance Brady Array | Isopeistic Fett-Vos |
			Humidity Indicator	Humichek (Beckman)	Hygrometer (Hygrodynamics)			
$MgCl_2$	0.33	0.32	NT	0.31	0.32	BR	0.31	BR
$Mg(NO_3)_2$	0.54	0.53	0.52*	0.53	0.46	BR	0.50	BR
$(NH_4)_2SO_4$	0.81	0.80	NT	0.80	0.79	0.78	0.80	0.84
$CdCl_2$	0.82	0.82	0.86*	0.81	0.83	0.83	0.79	0.83
Li_2SO_4	0.85	0.84	0.86*	0.84	0.83	0.83	0.86	0.87
Na_2HPO_4	0.96	0.96	NT	0.93	0.96	0.95	0.89	BR
K_2SO_4	0.97	0.97	0.96*	BR	0.97	0.99	0.98	BR
Average difference from theoretical a_w		0.01	0.02	0.02	0.02	0.02	0.03	0.02

NT = not tested
* = based on a single determination
BR = beyond range of the method used

TABLE 8

A$_W$ RESULTS FOR FOODS AND FOOD SYSTEMS

Material	Theoretical a$_w$ (Table 4)	Direct VPM	Electric Hygrosensors			Sina-scope	Resonance Brady Array	Isopeistic Fett-Vos
			Humidity Indicator	Humichek (Beckman)	Hygrometer Hygrodynamics			
Dry soup mix		0.21	0.21*	0.26	0.23	BR	0.23	BR
Thuringer		0.95	NT	NT	0.93	0.95	NT	0.94
Cheese		0.97	0.92*	0.93	0.94	0.94	0.89	0.95
Bread		0.97	0.94*	0.93	0.95	0.95	0.92	0.95
IM Pet food		0.87	NT	NT	0.90	0.86	NT	0.90
Hennican	0.91	0.90	0.90*	NT	0.90	0.88	NT	0.90
Hennican + 2% glycerol		0.87	0.90*	0.88	0.86	0.88	0.89	0.89
Hennican + 2% propylene glycol + 2% butylene glycol		0.86	0.88*	0.87	0.87	0.87	0.90	0.89
Semolina + egg		0.93	0.92*	0.93	0.89	0.92	0.89	0.90
Protein medium	0.90**							
a.		0.88	NT	NT	0.91	0.90	NT	0.91
b.		(i)	0.90	0.89	0.92	0.90	0.87	(i)
Protein medium	0.93**							
a.		0.90	NT	NT	0.93	0.92	NT	0.91
b.		(i)	0.92*	0.92	0.93	0.92	0.91	(i)

NT – not tested; BR = beyond range; * = based on single determination; (i) = impossible due to foaming;
** = Troller (1971)

standards. The Fett-Vos method had, for only a small number of salts tested, an average deviation of 0.02. As noted, use of this method is limited to the higher a_w's.

The relative accuracy of these methods was based on comparison to literature values. There is an intrinsic error in this as the absolute a_w's of the slurries may not be what is listed as "theoretical". The reasons for this are:

1. The standard value was found by averaging values from the literature. Different values are reported for a particular salt and temperature.

2. The values in the literature were determined by different methods which have different accuracies. For instance, Wexler and Hasegawa (1954) used a dewpoint apparatus (± 0.03) while Rockland (1960) used an electric Hygrometer (± 0.015, Hygrodynamics Bulletin, #SB-20). The accuracy of the latter may in fact be poorer than reported.

3. The literature is cross referenced, giving values unequal weight depending on how often a particular author is cited.

4. The a_w's of the slurries are temperature dependent. Some are greatly influenced by changes in temperature and, as a rule, such changes should be avoided. For instance, $Zn(NO_3)_2$ slurry has an a_w of 0.38 at $20^{\circ}C$ and 0.31 at $25^{\circ}C$.

5. Investigators handle the literature values in different ways. If analysis was performed at $22^{\circ}C$, values at 20° and/or 25° have been averaged (depending on what is available), or values at only the temperature closest to that of analysis have been averaged, i.e. $20^{\circ}C$. Thus although one can state that the VPM is best, this study points out that perhaps some National Bureau of Standards sample should be made and all investigators should use a correction factor with their method for reporting a_w. In

addition, Table 6 points out the possible need for multiple determination with some of the methods.

A study made by the U.S. National Bureau of Standards comparing published results of various investigators found that % relative humidity (a_w x 100) values for salt slurries in the literature fell within a range of ±1.5% RH (±0.015 a_w) from the NBS data (Wexler and Hasegawa, 1954). Thus, to date only a qualified expression of a_w should be made. Using modern technology, an absolute a_w of saturated salt solutions should be established. Since most methods rely on the salts for calibration, a direct measurement of the vapor pressure of the saturated salt slurries would give the best results. The method of choice is the VPM, since it functions independent of calibration by saturated salt solutions and measures a static physical phenomenon.

The results for the foods are shown in Table 8. The data for high a_w samples are quite variable. The a_w of cheese was determined to be from 0.89 (Brady Array) up to 0.97 (VPM). The Hygrometer and the Sina-scope gave a value of 0.94. Bread also gave a wide range of values. Again the higher VPM value may be due to the problem of accurate temperature control and monitoring in the high a_w range (Labuza, 1974). The Hygrometer, Sina-scope and Fett-Vos method all gave a value of 0.95. This trend does not exist for all samples; however, all other samples have a_w's determined within a range of ±0.02. Thuringer was measured as a_w 0.95 (VPM and Sina-scope) and as a_w 0.94 and 0.93 respectively by the Fett-Vos method and the Hygrometer. The data for the IMF samples are closer than for the high moisture samples.

Some of these samples have humectants (glycols) added, but the levels are so small that there should not be any significant interference. The

amount of the most volatile glycol (1,3 butylene glycol) in a VPM sample

(2% of 30g) does not contribute significantly to the measured a_w by VPM.

Accumulation of glycols onto sensors can cause erroneous readings but the

desorption process used for all sensors in this study excluded that

source of error. Adsorption of glycols onto the cellulose used in the

Fett-Vos procedure could also be a source of error if the glycol was a

significant proportion of the composition. No such samples were tested in

this study. The glycols were present as only 2% of the composition. The

a_w values of the Hennican plus 2% glycerol were from 0.86 (Hygrometer)

to 0.90 (Brady Array), the same extreme values as the Hennican with less

humectant.

The bacteriological media, NZ-amine and PHP, was made up and analyzed

on two occasions to compare reproducibility of preparation. Both trials

were analyzed by the Hygrometer and Sina-scope. Excessive foaming during

evacuation made the analysis difficult to impossible for the VPM and the

Fett-Vos method. Only the first trial was successfully analyzed by those

two methods. The three instruments tested at M.I.T. were used only in the

second trial. There is good agreement between Trials 1 and 2 by the VPM.

The a_w of each system, however, varies depending on the method of analysis.

The 0.90 media was measured as 0.90 (VPM) and as 0.93 (Hygrometer). In this

case, the loss of moisture from the samples for VPM analysis could be

significant. Extensive degasing (3 min) of the media was required to produce

a sample that could undergo the VPM evacuation step. The VPM values,

therefore, may be low for these two systems.

In general, the data for the foods varied by ±0.02 a_w units depending

on the method of determination used, as was the case for the salt solutions.

The foods of higher a_w gave a wider range as the VPM data was always higher.

However, no method seemed consistently high or low or better by these comparisons. As seen, however, one would question the validity of literature results either reported to 3 places for a_w or of absolute values for lower a_w limits of microbiological growth.

Based on this, however, some recommendations can be made for a_w determination.

1. Equilibration at the temperature of analysis is a must. 24 hr is sufficient for most samples, but more time may be needed for heterogenous samples.

2. Moisture transfer during equilibration or analysis must be kept at a minimum, whether from the atmosphere, sample-jar head space, or from the frozen state.

3. Sample history must be known. Extremes in temperature must be avoided if not a part of a process parameter. This treatment may affect the water in the sample from an adsorption or desorption situation. Change in temperature can cause a cross-over from one branch of a hysteresis loop to the other, resulting in a change in a_w.

4. Sensors, when used, should be maintained under optimum (dry) conditions and calibrated on a regular basis. Equilibration of sensor with sample should be complete.

5. Standard salt slurries must be pure. Those at a_w >0.95 must prove free of microbial activity.

6. Constant temperature must be maintained during calibration of sensors. Slight temperature fluxuations may cause the vapor in high a_w salts to condense. The resulting a_w may then be between that of the slurry and that of water, 1.0. Other details for use of saturated salt slurries appear in a publication put out by Hygrodynamics (Bulletin #5).

7. Contamination of sensors must be avoided.

Once standard values for salt slurries are established, they may be used to set and maintain calibration of the variety of a_w or relative humidity measuring methods. Any of the methods tested could be used in determining a_w. A few are less expensive, faster and/or more accurate than others. Positive and negative aspects must be weighed when choosing a method for basic research or quality control use.

If regulations are made governing a_w limitations for a particular product, then processors should standardize the methodology used for that particular product. Only then can comparisons be made with accuracy of ± 0.01 between laboratories or processors. It would be good for the National Bureau of Standards to prepare these standards.

5. REFERENCES

Bennewitz, P.F. 1973. The Brady Array – A new bulk effect humidity
  sensor. Society of Automotive Engineers, Detroit, Mich., Booklet #730571.

Carr, D.S. and B.L. Harris. 1949. Solutions for maintaining constant
  relative humidity. Ind. Eng. Chem. 41: 2014.

Fennema, O.R., W.D. Powrie and E.H. Marth. 1973. Low-Temperature Preservation
  of Foods and Living Matter. Marcel Dekker Publishers, Inc., New York.
  page 128.

Fett, H.M. 1974. Water activity determination in foods in the range 0.80
  to 0.99. J. Food Sci. 38: 1097.

General Eastern Corp. 36 Maple St., Watertown, Mass. 02172. Data Bulletin
  for Relative Humidity Indicator, Models 400C and 400D, #400-0174.

Handbook of Chemistry and Physics. 1972-1973. R.C. Weast, editor. 53rd
  edition. Chemical Rubber Co., Cleveland, Ohio. pg. E-40.

Hygrodynamics Technical Bulletin #5. Creating and maintaining humidities
  by salt solutions. Hygrodynamics Inc., Silver Springs, Maryland.

Hygrodynamics Bulletin #SB-20. Humidity sensors. Hygrodynamics Inc.,
  Silver Springs, Maryland.

International Critical Tables of Numerical Data, Physics, Chemistry and
  Technology. 1926. E.W. Washburn, editor. 1st edition. McGraw-Hill
  Co., Inc. New York. pg. 67.

Labuza, T.P. 1974. "Sorption phenomenon in foods". In Theory, Determination
  and Control of Physical Properties of Food Materials. D. Reidel
  Publishing Co., Dordrecht, Holland. Chapter 10, pg. 119.

National Bureau of Standards. 1974. Technical Bulletin #NBSIR-74-477.

O'Brien, F. E. 1948. J. Sci. Instr. 25: 73.

Plitman, M., Y. Park, R. Gomez and A.J. Sinskey. 1973. Viability of
  Staphylococcus aureus in intermediate moisture meats. J. Food Sci.
  38: 1004.

Richardson, G.M. and R.S. Malthus. 1955. Salts for static control of
  humidity at relatively low levels. J. Appl. Chem. 5: 557.

Rockland, L.B. 1960. Saturated salt solutions for static control of
  relative humidity between 5° and $40^\circ C$. Anal. Chem. 32: 1375.

Sina Instruction Manual, Bulletin #16-104-3, 22-102-1, 22-102-2. Limited
    Co. for instrumentation and automatics. Zurich, Switzerland.

Sood, V.C. and D.R. Heldman. 1974. Analysis of vapor pressure manometer
    for measurement of water activity in non-fat dry milk. J. Food Sci.
    39: 1011.

Stokes, R.H. and R. Robinson. 1949. Ind. Eng. Chem. 41: 2013.

Taylor, A.A. 1961. Determination of moisture equilibrium in dehydrated
    foods. Food Technol. 15: 536.

Troller, J.A. 1971. Effect of water activity on enterotoxin B production
    and growth of Staphylococcus aureus. Appl. Microbiol. 21: 435-439.

Vos, P.T. and T.P. Labuza. 1974. Technique for measurement of water
    activity in the high a_w range. J. Agric. Food Chem. 22(2): 326.

Wexler, A. and S. Hasegawa. 1954. Relative humidity - temperature relation-
    ships of some saturated salt solutions in the temperature range 0°
    to 50°C. J. Res. N.B.S. 53: 19.

Wink, W.A. and G.R. Sears. 1950. Equilibrium relative humidities above
    saturated salt solutions at various temperatures. TAPPI 33(9): 96A.

F. Summary and Recommendations

With respect to water binding agents and their properties:

1. Salts have the greatest water holding capacity due to their low molecular weight.

2. Of the liquid glycols, glycerol shows the best water holding capacity. This is advantageous in addition to its inhibition of browning.

3. Of the sugars, sorbitol has the best water binding properties. This is significant because it is also useful in inhibiting browning.

4. Propylene glycol holds 40% less water than glycerol but may be preferred as a humectant because it specifically inhibits mold and staphylococcal growth as well as inhibits non-enzymatic browning in intermediate moisture foods.

5. There is no effect of the method of mixing of humectants into an IMF system on the degree of a_w lowering.

6. The Ross equation and the derived linear slope method give very accurate prediction of water activity lowering for humectants.

7. With respect to a_w measurement the vapor pressure manometric technique is the best available.

8. Standards for saturated salt solutions should be prepared to ensure a_w comparison between all laboratories.

9. Most electric hygrometer sensing devices cannot measure a_w accurately so the values should be reported only to two decimal places with a range of ± 0.02.

VI   Accelerated Shelf Life Testing of Antioxidants in

Intermediate Moisture Systems

A.   Introduction

As noted in the introduction section of this report,
rancidity is one of the major deteriorative reactions occurring
in IM food systems if they contain unsaturated fats. This leads
to rapid production of off odors and flavors and unacceptability
of the product. In the previous studies under this contract
it was found that:

1.   Antioxidants will afford some degree of protection
to rancidity in IMF. Of the primary antioxidants, BHA and BHT
(at the 100ppm level) were the most effective, giving protection
factors (increased induction time) of 2-3 times in the IM range.

2.   Packaging systems that lead to zero oxygen levels,
such as the Miraflex 7F   (American Can Company) give the best
protection, however the cost may be prohibitive as compared to
that of using antioxidants.

Because oxidation is relatively slow at room temperature
in most real food systems, it would be useful to develop some
accelerated shelf life tests at high temperature. Much work has
been done using high temperature - high pressure oxidation
for study of stability of oils used in food processing. The
major works have been done by Pohle et al (1962, 1963, 1964),
Bennett and Byer (1964) and Berger (1971). Most of these tests
involve oxidation of oils at 80-100°C with oxygen pressures

ranging from 0.21 atm up to 100 psi. These tests include:

a. AOM: Here air is bubbled through oil at 2.33 cc/sec at
98°C. Measure time needed for peroxide value to reach 100.

b. ASTM-AOCS procedure: In this test an oxygen bomb is
used. The initial oxygen pressure being 50 psi, the temperature
100°C. Endpoint is reached when the pressure drops by 2 psi/h.

c. Ecky Manometric: 1 gram oil is deposited on 12.5 g
clean sand at 80°C. System attached to manometer. Measure time
for pressure to drop by 40 mm Hg.

d. Schaal Oven Test: Here the sample is held in closed
containers at 140°C and is smelled periodically. Measure time
for organoleptic score to reach 4.5.

In addition there are different variants of each method,
each using different endpoints as well as conditions of tempera-
ture and pressure, etc.

Pohle et. al. (1964) used five methods (including the AOM,
modified ASTM, Schaal Oven Test at 60°C) to test stability of
three types of fat concurrently. They ran storage tests at
30°C. When they plotted the induction period at 30°C (the time
required by a sample to develop slight to moderate off-odor,
i.e. a flavor score of 4.5) vs. induction period at 100°C (the
time required for pressure to drop by 2 psi/hr) in the Oxygen
Bomb Test, they found that the data points fell on three distinct
lines (although of course there was considerable scatter). There
was one line for each type of fat. The points nearest to the
origin of the graph represented the controls, those points
farther away were for those samples containing antioxidants.

Thus, 1 hr at 100°C in the oxygen bomb was equivalent to 2, 5.7 and 17.5 days of storage time at 30°C for tallow, hydrogenated vegetable oil and lard respectively.

What is interesting about these results is not the numbers obtained, but the fact that the addition of antioxidants did not affect the relationships obtained. The ratio between the rate at 30°C and the rate at 100°C and hence the "apparent" activation energy of the overall reaction does thus not seem to be greatly affected by the addition of the antioxidants. However, for the three types of fats in question, greatly different values were obtained for the "apparent" activation energy. Unfortunately, the workers did not give an accurate product specification of the fats used. Also their definition of induction period is arbitrary which tends to annihilate the significance of the results obtained. This work is, however, one of the few that has attempted to correlate data obtained by the Oxygen Bomb Test (probably the best of the rapid test procedures developed to date) with actual storage life data.

In general the different test procedures did not correlate among themselves, which is not surprising, since all are based on empirical endpoints and conditions.

The problem with all these methods, is that the high temperature causes other reactions to proceed and interfere; the fat may be melted out of the product giving erroneous results and at high temperature and pressure, the water activity of the product is not the same as under normal storage conditions.

In fact, in order to get meaningful results the temperature should not exceed 45-50°C. For certain real foods, especially IMF, where oxidation is a major problem, such a moderate temperature elevation is often adequate to accelerate the reaction sufficiently so that data can be obtained in a short span of time.

Considerable work has been done in this department on antioxidant effectiveness in IM range. Chou and Labuza (1975) using a microcrystalline cellulose - glycerol - methyl linoleate model system tested the effectiveness of various antioxidants in the IM range. In their system I 100 ppm Co(II) [lipid basis] was added. BHA and BHT both gave considerable protection (protection factor, PF = 2-3). In their system II 1000 ppm [lipid basis] of mixed metals were added. In this mixture only Fe(II) (100 ppm) and Cu(II) (5.3 ppm) are expected to be effective catalysts. In this system EDTA, at a very low level (0.08 M/M metal) gave good protection, PF ranging from 4-10 depending on conditions. BHA (200 ppm) again was very effective (BHT not tested). The protective effect of citric acid and isopropyl citrate (both at 0.5 M/M metal) and α-tocopherol was slight to moderate.

These data show that under the test conditions used, EDTA is a much better antioxidant than citric acid and its fat soluble analogue, isopropyl citrate. This is not unexpected since EDTA is known to bind all metal ions (except alkali metals) and binds Fe(II), Co(II) and Cu(II) very tightly. What is surprising, however, is that EDTA should be able to do this

at a level of only 0.08 M/M metal.

Labuza et al (1971), using a similar model system containing 100 ppm cobalt (lipid basis) at a_w 0.6, found EDTA and citric acid (10 M/M metal) to be very potent, α-tocopherol (200 ppm) gave very little protection and ascorbic acid (300 ppm) was an effective prooxidant. When a protein was added to such a system and a_w adjusted to 0.75 propyl gallate and BHT (at 200 ppm) offered no protection, whereas BHA (200 ppm) and EDTA (10 M/M metal) were very effective. Finally in a system containing chicken meat, glycerol and cellulose, EDTA (400 ppm) offered moderate protection whereas BHA (200 ppm) was a very powerful antioxidant.

These data confirm the strong protective effect of EDTA in model systems. Two points should be noted, however; first, that the levels used were extremely high; secondly, that the EDTA is free to mix in with the lipid and aqueous phases and so can pick up most of the metal ions. In the chicken system, on the other hand, the EDTA will not be able to fully penetrate the fat in the same manner.

Considerable work has been done in the past on developing reliable methods for determining rancidity. One of the best methods is the iodometric peroxide method. A good deal of time and effort has been spent on finding the best conditions for the peroxide value determination. Link and Formo (1961) have reviewed some of the methods including this one. They point to seven references that indicate that the use of small sample size gives rise to marked rise in peroxide value. Lea (1941),

however, got best results using a small, 1 g sample size.
Link and Formo also discuss the effect of oxygen on the peroxide
value and conclude that an inert atmosphere is essential. A
recent paper by Fiedler (1974) brings one to the same conclusion.
She shows that the key variables in this test are: $N_2(g)$-
bubbling, sample size, temperature and time.

Based on these considerations the specific objectives of
this work were twofold:

(i) to examine the value of using accelerated storage
life tests at 45^oC to predict actual shelf life at normal storage
temperatures, using both model systems and actual foods in
which the critical mode of deterioration is lipid oxidation.

(ii) to test the relative effectiveness of various anti-
oxidants in extending the shelf life of model and real food
systems.

In view of some technical difficulties reported in the
literature with the peroxide value method, some time was spent
on looking at the effect of several critical variables on results
obtained with this method.

B   Materials, Methods and Calculations

1. Chemicals used

(1) Microcrystalline cellulose (Avicel®), FMC Corp-
oration, PH-101.

(2) Methyl linoleate, $CH_3OOCC_{17}H_{31}$, NU-CHEK-PREP, Inc.,
99%+.

(3)   Glycerol, $C_3H_8O_3$, AR, Mallinkrodt, 5092, 95%+.

(4)   Coboltous nitrate, $CO(NO_3)_2 \cdot 6H_2O$, A.C.S., Matheson, Coleman & Bell, CB317.

(5)   Potassium iodide, KI, A.C.S., Fisher Scientific Co., P-410.

(6)   Sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$, A.C.S., Fisher Scientific Co., S-445.

(7)   Potassium oxalate, $K_2C_2O_4 \cdot H_2O$, AR, Mallinkrodt, 7052.

(8)   Starch soluble, AR, Mallinkrodt, 8183.

(9)   Potassium dichromate, $K_2Cr_2O_7$, AR, Mallinkrodt, 6770.

(10)   Glacial acetic acid, CH_3COOH, J.T. Baker Chem. Co.,3-9507.

(11)   Chloroform, $CHCl_3$, A.C.S., Fisher Scientific Co., C-298.

(12)   Methanol, CH_3OH, A.C.S., Matheson, Coleman & Bell, MX485, CB522.

(13)   dl-Alpha tocepherol [vitamin E], $C_{28}H_{48}O_2$, NF-FCC, Roche, Lot No. 205032, obtained in May 1972.

(14)   Isopropyl citrate, IPC, 60% monoester $C_9H_{14}O_7$, obtained in 1972.

(15)   Tertiary butylhydroquinone, TBHQ, $C_{10}H_{14}O_2$, Eastman Chemical Products, Inc., obtained in March 1975.

(16)   Propyl gallate, PG, $C_{10}H_{12}O_5$, Eastman Chemical Products, Inc., obtained in March 1975.

(17)   Butylated hydroxyanisole, BHA, $C_{11}H_{16}O_2$, Eastman Chemical Products, Inc., obtained in March 1975.

(18)   Butylated hydroxytoluene, BHT, $C_{15}H_{24}O$, Eastman Chemical Products, Inc., obtained in March 1975.

(19)   Ethylenediaminetetraacetic acid, EDTA, $C_{10}H_{16}O_8N_2 \cdot Na_2 \cdot 2H_2O$, Eastman Chemical Products, Inc., obtained in 1973.

(20)   L-(+)-ascorbic acid [vitamin C], $C_6H_8O_6$, Eastman Chemical Products, Inc., obtained in June 1973.

(21)   Ferrous sulfate, $FeSO_4 \cdot 7H_2O$, A.C.S., J.T. Baker Chemical Company, 2070.

(22)   Sodium carbonate, Na_2CO_3, A.C.S., Matheson, Coleman & Bell, CB705.

(23)   Distilled, deionized water was used throughout study.

(24)   Nitrogen, N_2, Chemetron Corp., National Cylinder Gas Div.

(25)   Mazola corn oil (pure, sample obtained 8/2/1973), Best Foods, A Division of CPC International, Inc..

2.   Calibration of Manometer Flasks and Manometers

The method of Grisolia (Umbreit et al., 1964) was followed whereby the total flask volume (TV) is determined using liquid mercury.

3.   Microcrystalline Cellulose Preparations

The cellulose was impregnated with catalyst as follows:
A known amount of either Fe(II) or Co(II) standard solution was added to approximately 3 l of water. Then 1000 g of cellulose was

gradually added. The slurry was stirred with 8 mm glass rod
to a uniform consistency, poured into stainless steel trays,
covered with Al-foil, frozen at -29°C overnight, covered with
cheese cloth and freeze-dried for 72 h. After grinding the
freeze-dried mixture, the cellulose was ready for use.

The following mixtures were made up: cellulose containing:
10 ppm Fe(II) [49.78 mg $FeSO_4 \cdot 7H_2O$ per kg cellulose], 100 ppm
Fe(II) [497.82 mg $FeSO_4 \cdot 7H_2O$ per kg cellulose], containing 100 ppm
Co(II) [493.87 mg $Co(NO_3)_2 \cdot 6H_2O$ per kg cellulose].

4. Model System Preparation

Glycerol and the cellulose were dried over $CaSO_4$ for
several days in evacuated desiccators. The main sources of moist-
ure in the dry system were the glycerol and moisture pick-up
during the system preparation. The antioxidant solutions were
prepared the day preceding each run. The solvent used was meth-
anol, except for EDTA (water). The concentrations were 10 mg
antioxidants/ml and during the mixing enough was added so the
lipid phase would contain 200 ppm of the antioxidant (table 2).

The composition of the systems used is shown in Table 1.
First the methyl linoleate and the antioxidant solution were
mixed (pure methanol was added to the control), then the
glycerol, the water and finally the cellulose were added to
the mixture. The systems were thoroughly stirred in amber jars
in a 2-4°C cooler and were subsequently transferred to 250 ml
round-bottom flasks fitted with glass stoppers.

Table 1

COMPOSITION AND DENSITY OF DRY AND IM SYSTEMS

Component	Wt % in Dry System	Wt % in IM System	Density
Avicel	49.86	38.40	1.35
Glycerol	39.89	30.72	1.252
Water	0.10	23.06	1.000
Methyl linoleate	9.97	7.68	0.889
Methanol	0.18	0.14	0.78
Fe(II)	0.00050	0.00038	
Antioxidant	0.0020	0.0015	
	100.00	100.00	
Overall Density	1.264.00	1.200.00	

Table 2

ANTIOXIDANT CONCENTRATIONS

	TBHQ	AA	IPC	EDTA	E	BHA	BHT	PG
MWt	166	176	318	372	416	180	220	212
ppm AO(lipid base)	200	200	200	200	200	200	200	200
mmoles AO/kg lipid	1.21	1.14	0.854	0.54	0.48	1.11	0.91	0.94
moles AO/mole metal	1.35	1.27	0.704	0.60	0.54	1.24	1.02	1.05

AO = antioxidant

TBHQ = Tertiary butylhydroquinone

AA   = Ascorbic acid

IPC   = Isopropyl citrate

EDTA = Ethylenediaminetetraacetic acid

E   = Vitamin E, dl-Alpha tocopherol

BHA   = Butylated hydroxyanisole

BHT   = Butylated hydroxytoluene

PG   = Propyl gallate

The water activity of the dry system was somewhat variable
and ranged from 0.08 to 0.15 at the start if each run. The
water activity of the IM system was consistently 0.74-0.76.

5. Manometric Determination of Oxygen Absorption

As soon as the mixture was ready, 4.00 g (solid
basis) samples were added to each of two or three Warburg
flasks. The flasks were connected to the appropriate manometers
and taken into the environmental room (temperature T_1), placed
in the Warburg water bath, also adjusted to temperature T_1, i.e.
25, 35 or 45°C. The bath was then covered with black cloth.
After 10 minutes equilibration time the level of the manometers
was adjusted to approximately 150 manometric units. Frequent
measurements of manometric values were made with time. When
the level in the left arm of the manometer dropped below 100
manometric units (mm), flasks were taken out of the bath, dried
and opened. Either 30 sec equilibration with the air in the room
was allowed or the flasks were flushed with either dry or 0.75
a_w air. Thermobarometers containing no lipid, but all other
system ingredients in same proportions as the test systems were
made up and used to correct for environmental changes.

6. Peroxide Value Determinations

a. General Procedure

The round-bottom flask containing the remainder
of the mixture was stoppered, covered with Al-foil, placed
in water bath (at T_1) in the environmental room. Samples

ORIGINAL PAGE IS POOR

containing 0.1 g lipid were collected at regular intervals and analysed using the following method:

25 ml of chloroform-methanol solution was added to the sample in a 125 ml Erlenmeyer flask. The solution was flushed with $N_2(g)$ and the stoppered flask placed on a shaker for 30 min. Contents were filtered through Whatman No. 50 filter paper, using a Buchner funnel. Filtrate was evaporated to dryness in a Büchi rotary evaporator. Bath temperature was 45^oC. Vaccuum was broken with $N_2(g)$. Under constant flushing with $N_2(g)$, 10 ml of 3:2 glacial acetic acid: chloroform solution was added. Then 0.5 ml of a freshly made saturated aqueous solution of potassium iodide was added and exactly two minutes later 15 ml of water were added to stop the reaction. The reaction temperature was approximately $10-15^o$C. The mixture was titrated with prestandardized 0.01 N $Na_2S_2O_3$ solution. No correction factors for sample size were used.

b. Preparation of Sodium Thiosulfate Solution

Three to four liters of water were boiled for 5 min and then cooled to room temperature. 2.5 g of $Na_2S_2O_3 \cdot 5H_2O$ and 0.1 g Na_2CO_3 were added for every liter of water used. When solution was complete, the clear solution was poured into a clean screw-capped amber jar and stored in the cooler. This method allows maximum protection against bacterial growth and chemical changes in the solution. The solution was frequently standardized with $K_2Cr_2O_7$.

c. Preparation of Starch

Starch indicator solution was freshly made up at frequent intervals as follows: A paste was made by rubbing 3 g soluble starch in 30 ml boiling water. The slurry was poured into a beaker containing about 100 ml boiling water. 0.5 ml was used per titration with indicator introduced towards the end of the titration, especially when the I_2 concentration was high.

d. Method Variabilities

The effect of several variables on the PV determination was investigated using rancid corn oil as substrate. Except for the changes listed, the above method was closely followed. Since no extraction was needed, the 3:2 solution was added directly to the oil sample.

(i) Effect of Nitrogen Bubbling

1.00 g samples were used. Three variants were compared. $N_2(g)$ was allowed to bubble through the corn oil, 3:2 solution, KI-solution prior to mixing as well as through the reaction mixture during titration. In another, $N_2(g)$ was bubbled only through the 3:2 solution for 20 seconds prior to adding the KI-solution as well as during the reaction. In the third, no nitrogen was added at all. Instead the reaction flask was swirled gently during the reaction to allow more oxygen to dissolve in the mixture.

(ii) Effect of Reaction Time

Reaction times of 1.0, 1.5, 2.0 and 3.0 min were used. The sample size was 1.0 g and reaction temperature

15°C. Duplicate determinations were made for each time setting. $N_2(g)$-bubbling was used during the reaction.

(iii) Effect of Reaction Temperature

The reaction was thermostated at three different temperatures: 15, 25 and 35°C. 1.0 g samples were used. Nitrogen was added during the reaction. At least duplicate determinations were made at each temperature.

(iv) Effect of Sample Size

Five different sample sizes, 0.1, 0.25, 1.0, 3.0 and 5.0 g ($\pm$ 0.005 g) were used. $N_2(g)$-bubbling was used throughout and the reaction temperature was 18°C. At least duplicate determinations were made for each sample size.

(v) Precision of Method

$N_2(g)$-bubbling was used throughout. The reaction was thermostated at 18°C. 0.25 g ($\pm$ 0.005 g) samples were used. Seven PV determinations were made on the corn oil.

7. Water Activity Measurements

The standard V.P.M. technique was used to measure water activities of the system (see section V).

8. Model System Studies

Two systems were used (dry and IM). Their composition and total densities are listed in Table 1. On a dry weight basis

their composition is identical. Results from runs 1-11 are
included in this report. Except for run 1, 50 ppm (lipid
basis) of Fe(II) was used as prooxidant throughout. The
overall characteristics (temperature, a_w, antioxidants) of
runs 1-11 are listed in Table 3. Other procedural details are
listed in Table 4. As shown, various modifications of the PV
and oxygen absorption method were made during the study.

System preparation took place in the cooler. Water
activity measurements were made at room temperature. Oxygen
uptake studies were performed in the environmental room.
Peroxide value studies were performed in this room also and
emphasis was placed on storing and handling PV samples in the
same manner as samples used to measure oxygen uptake. The
peroxide value titrations were carried out at room temperature,
however.

9. Calculations

A brief outline of the calculations used in this
study are given below. A Compucorp 326 Scientist Computer
(Computer Design Corporation) was used to analyse the data.

a. Oxygen Uptake Studies

The data obtained in runs 1-11 were analysed as
follows: An average k-value for the two or three Warburg
flasks was calculated for each sample, using the following
formula:

$$k = \frac{(TV - SV)\ 273.000 \times 2.5}{760T} = 2.915\ (TV - SV) \qquad (1)$$

Table 3

GENERAL CHARACTERISTICS OF RUNS 1-11

Run No.	Temperature °C	a_w	Antioxidants
1	35	0.11	BHA, BHT, PG, TBHQ
2	35	0.11	BHA, IPC
3	35	0.11	E, PG, TBHQ
4	35	0.11	BHA, BHT, E, IPC, TBHQ
5	35	0.11	None
		0.75	None
6	35	0.75	BHA, BHT, E, IPC, PG, TBHQ
7	25	0.75	BHA, BHT, E, EDTA, PG
8	25	0.11	BHA, BHT
		0.75	BHA, BHT
	45	0.75	BHA, BHT
9	45	0.75	AA, BHA, BHT, EDTA, PG
10	45	0.11	AA, E, EDTA, PG
		0.75	AA, E, EDTA, PG
11	45	0.11	AA, EDTA, PG
	45	0.75	AA. EDTA, PG

---

* For antioxidant abbreviations, see Table 3

Table 4

EXPERIMENTAL DESIGN OF RUNS 1-11

Design Features	1	2	3	4	5	6	7	8	9	10	11
ppm Fe(II)[lipid basis]	500	50	50	50	50	50	50	50	50	50	50
control tested	+	+	+		+	+	+	+	+	+	+
initial a_w tested	+	+	+			+	+	+		+	+
final and initial a_w tested				+	+				+		
controlled R.H. air used				+	+	+	+	+	+	+	+
Warburg bath covered		+	+	+	+	+	+	+	+	+	+
Warburg flasks kept in:											
water bath at T_1	+	+	+	+	+	+	+			+	+
air at T_1								+	+		
PV samples contained in:											
open beaker over salt	+										
capped amber jars		+	+	+	+	+	+	+			
round-bottom flasks(foil covered)										+	+
PV flasks kept in:											
water bath at T_1										+	+
air at T_1	+	+	+	+	+	+	+	+	+		

where k = flask constant which converts from manometric units [MM Hg] to oxygen uptake per gram lipid [μL O_2(STP)/G LIPID]

TV = total volume of flask and stem (ML)

SV = sample volume (wet basis) (ML) - listed in Table 5

T = temperature of environmental room = 308°K

Sample calculation: Given the following data for a dry system after, say, 20 hr under certain conditions:

Flask No.	1	2	3	Average
TV(ML)	24	25	26	25
Δh (mano-metric units: MM HG	90	100	110	100

From Table 5 we find the sample volume, SV for the dry system = 3.17 ML and hence, (ignoring thermobarometer changes):

$$k_{av} = 2.915 \cdot (25.00 - 3.17) = 63.63 \ \mu L \ O_2(STP)/G \ LIPID - MM \ Hg$$

Now oxygen uptake (μL O_2(STP)/G) = $\Delta h \cdot k_{av}$ = 63.63$\cdot$100 = 6363

This is equivalent to 8.36% of the linoleate being oxidized

b. Peroxide Value Studies

Similarly the oxygen uptake can be calculated from the peroxide value if we assume (1) that all the oxygen absorbed is used to form monohydroperoxides and (2) that decomposition of hydroperoxides is not significant. The peroxide value is determined by $S_2O_3^{=}$ titration. The conversion factor used to convert from peroxide values (MEQ HYDROPEROXIDE/KG LIPID) to μL O_2(STP)/G LIPID is 11.2. Since (N) x (ML) = (MEQ) we get:

Table 5

FIGURES FOR WARBURG SAMPLE VOLUME

Parameter	Dry System	IM System
G SYSTEM/WARBURG FLASK [G]	4.00	5.20
DENSITY [G/ML]	1.264	1.200
SAMPLE VOLUME, SV [ML]	3.165	4.333

Oxygen uptake $(\mu L\ O_2(STP)/G) = 11.2 \cdot \dfrac{(ML\ S_2O_3^=)(N_{S_2O_3^=})}{(G\ LIPID/SAMPLE)} \cdot 10^3$ (2)

Sample calculation: Assume that for the example given above
we need 5.75 ml of 0.0100 N $S_2O_3^-$ to titrate a 0.100 g sample
of the lipid. Then

Oxygen uptake $(\mu L\ O_2(STP)/G) = \dfrac{11,200 \cdot 5.75 \cdot 10^{-2}}{0.100} = 6440$

The two values, therefore, are comparable confirming the
assumptions made.

C.   Results

1.   PV Method Studies

The results in Table 6 indicate the effect of using an
inert environment during the determination. When no N_2 was used,
PV up to 20% or higher were obtained as compared to the values
obtained using nitrogen. About one fourth of this difference
stems from the fact that as N_2 is allowed to bubble through the
reaction medium, the temperature dropped by approximately 8^oC
(see effect of temperature below). No significant difference
was seen between results obtained for determinations when N_2
was used throughout as compared to using nitrogen only during
the reaction. Clearly it is of great importance to keep oxygen
away from the reaction medium during the reaction between hydro-
peroxides and the iodide ions.

Figure 1 a shows the effect of reaction time on the peroxide
value (sample size: 1 g). It should be noted that after 2 min,

Table 6

EFFECT OF FLUSHING WITH NITROGEN GAS

No	$N_2(g)$ - Flux	Mls.0.01018 N S_2O_3	P.V.
1	During reaction	7.55	74.1
2	"	7.40	72.7
3	Throughout	7.60	74.7
4	"	7.68	75.4
5	None	9.00	88.4
6	"	8.90	87.4

Sample size = 1.00 grams

Reaction temperature = 15^oC for no. 1-4 and 23^oC for no. 5 and 24^oC for no. 6
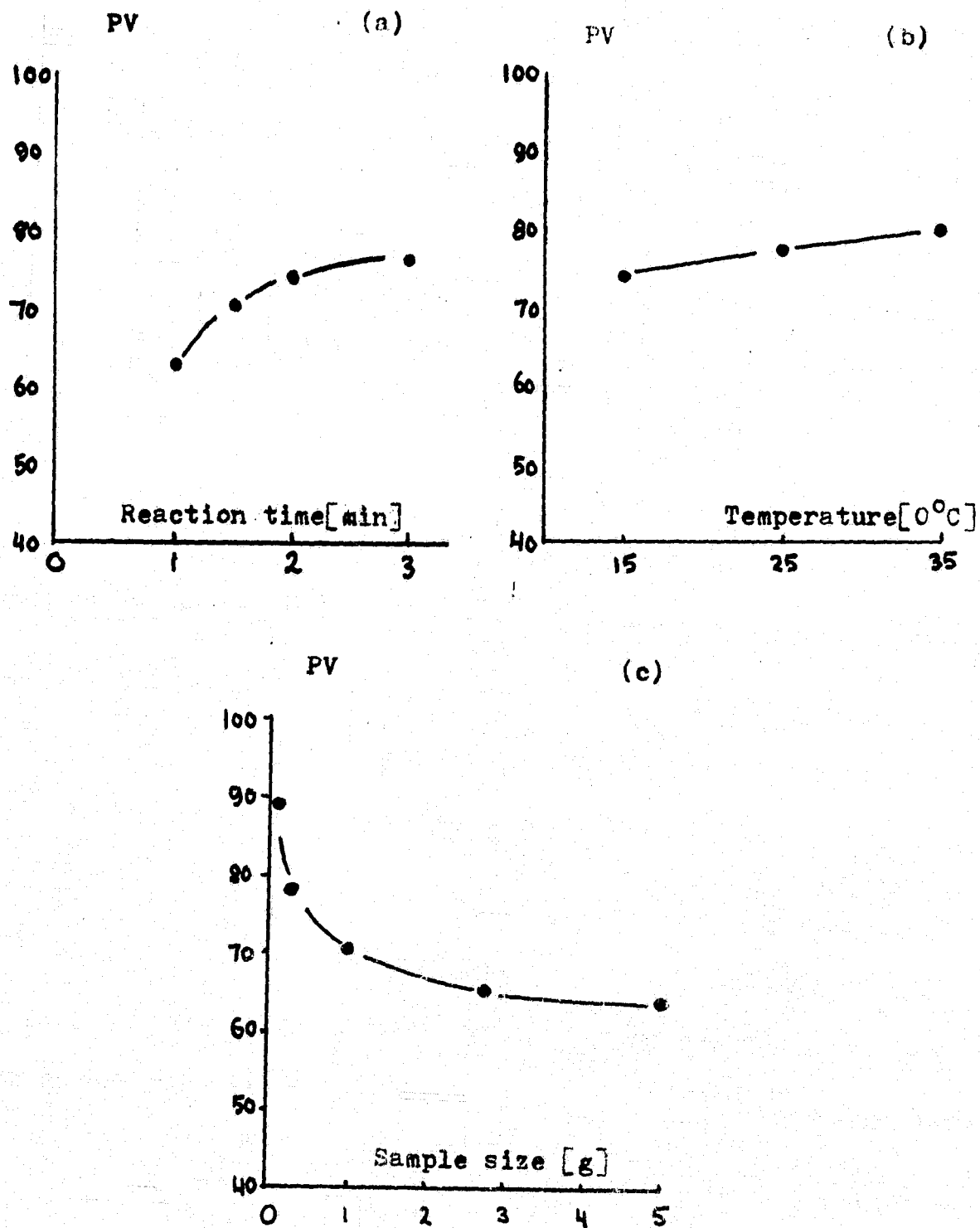
Reaction time = 2.0 min.

Figure 1   EFFECT OF SOME KEY VARIABLES ON PV

the reaction time normally allowed, not all the hydroperoxides have reacted. Thus another error (although seemingly small, of the order of 5%) is introduced. When precise comparisons between oxygen uptake and peroxide value are to be made, it may be advisable to allow the reaction to proceed for a longer period. If a two minute reaction time is to be used, accurate timing is necessary.

Figure 1 b indicates the effect of temperature on the peroxide values obtained. It is clear that as the temperature is increased from 15 to 25°C, the peroxide value obtained increases by about 5%. This is not a great difference, but enough to call for some control over this variable. No more than $2-3^{\circ}$C temperature fluctuations should be allowed during the reaction.

Figure 1 c shows effect of sample size on peroxide value. It is clear that in this system the peroxide value does not stay constant, but rather decreases sharply with sample size. The most reasonable explanation is that a constant error is operating and that a fairly large sample size is needed to overcome this. Alternatively, the larger samples need more than 2 minutes for stoichiometric reaction between peroxides and I^-.

In order to check the reliability (precision) of the method 7 determinations were carried out using standardized conditions. The results are shown in Table 7 (N_2-bubbling and thermostating). As indicated, the average PV is 81.1 with standard deviations of about 1.5%. This error is at least

Table 7

RELIABILITY OF PEROXIDE VALUE DETERMINATION

No.	Mls. 0.0100 N S_2O_3	P.V.
1	2.04	81.6
2	2.06	82.4
3	2.00	80.0
4	2.04	81.6
5	2.00	80.0
6	2.04	81.6
7	2.01	80.4

Sample size = 0.25 grams

$N_2(g)$ used throughout

Reaction temperature = $18^{\circ}C$

Reaction time = 2.0 min

partly caused by relatively inaccurate electronic balance ($\pm$ 0.005 g) used for sample weighing. The method is therefore precise within these limits.

In conclusion, N_2-bubbling during the reaction, accurate timing, no more than 2-3°C temperature fluctuations during the reaction and great caution when scaling down sample sizes are important factors contributing to the accuracy of the method. It should be emphasized, however, that pure, but highly rancid corn oil was arbitrarily chosen for this study and may not be representative of assay conditions obtained in model systems.

2. Model System Studies

Table 8 indicates results of a_w determinations in runs 1-11. The data show that the dry system is very prone to changes in a_w (see data from runs 4 and 5). By using dried air for flushing and later using glass-stoppered containers this problem was eliminated. In the IM region the a_w was fairly constant.

In this work it was decided to use iron rather than cobalt as catalyst. Whereas Co is found in extremely low concentrations in foods iron is ubiquitous in a wide variety of foods. The average Fe-concentration in consumed foods is 90-100 ppm [lipid basis] making it the most important catalyst of lipid oxidation in foods.

Figure 2 shows results of run 1 using a dry system [500 ppm Fe or Co (lipid basis)]. Note the much greater potency of the iron. Clearly this Fe(II)-level is too high

Table 8

RESULTS OF a_w MEASUREMENTS

Run No.	System in which a_w Measured	a_{w1}	a_{w2}
1	Dry Fe(II)-control	0.13	
2	Dry Fe(II)-control	0.09	
	Dry Fe(II)-BHT	0.10	
3	Dry Fe(II)-BHT	0.11	0.12
	Dry Fe(II)-E		0.14
	Dry Fe(II)-TBHQ		0.16
	Dry Fe(II)-PG		0.13
4	Dry Fe(II)-control	0.08	0.15
	Dry Fe(II)-E	0.10	0.10
	Dry Fe(II)-TBHQ	0.08	0.13
	Dry Fe(II)-PG	0.07	0.11
	Dry Fe(II)-IPC	0.10	
5	Dry Fe(II)-control	0.17	0.14
	IM Fe(II)-control	0.76	0.76
6	IM Fe(II)-control	0.74	
	IM Fe(II)-PG	0.78	
	IM Fe(II)-TBHQ	0.76	
7	IM Fe(II)-control	0.75	
8	Dry Fe(II)-BHA	0.17	
	Dry Fe(II)-BHT	0.15	
	IM Fe(II)-BHT	0.75	
9	IM BHT	0.75	0.68

Figure 2   RESULTS OF RUN 1 - WARBURG DATA

Legend (as shown in figure):

Fe(II)-CONTROL(DRY)
Fe(II)-TBHQ(DRY)
Fe(II)-PG(DRY)
Fe(II)-BHA(DRY)
Fe(II)-BHT(DRY)
Co(II)-CONTROL(DRY)
Co(II)-CONTROL(IM)

Temperature of run = 35°C

3 % OXIDATION LEVEL

[μL O₂(STP)/ G LIPID]·10⁻³

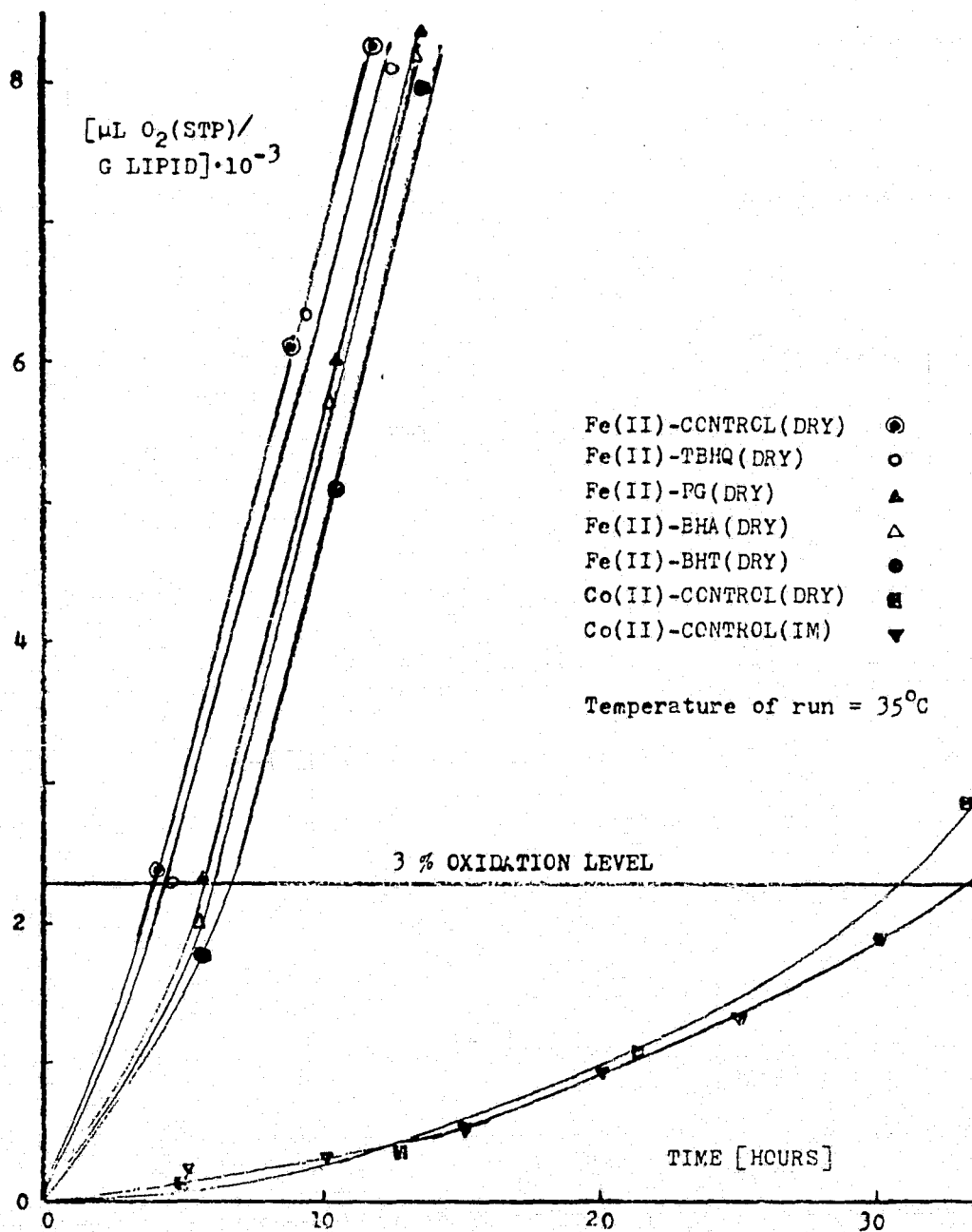TIME [HOURS]

Since none of the antioxidants used afforded any appreciable protection. However, a slight protective effect was seen to increase in the order: TBHQ, PG, BHA, BHT. It can be noted that coincidentally the dry Co(II) control and the IM Co(II) show comparable rates of lipid oxidation. It should be brought out in regard to run 1 that the Warburg samples were exposed to fluorescent light. It was later found that light was a factor in accelerating the lipid oxidation in this test system. As a result, the data presented in figure 2 are subject to a certain error.

It was noted towards the end of this experiment (the beakers and Warburg samples were left in the room for about 5 days) that a red discoloration occurred in the samples stored in open beakers in the 11% RH desiccator over LiCl solution provided the samples contained both lipid and Fe(II). This color formation did not occur in the Warburg flasks indicating that possibly the salt solution may in some way be involved.

It was clear from this run that (1) a lower Fe(II) concentration had to be used and (2) that the samples should be kept in tightly capped containers. In all subsequent runs 50 ppm Fe(II) (lipid basis) was used, making the molar ratio of antioxidant to metal close to 1 (see Table 2).

The results in figure 3 show a typical oxygen uptake curve. In this case the antioxidant used was tertiary butylhydroquinone (TBHQ) in a dry system at 35°C. Note the close correspondence of oxygen uptake and peroxide value when calculated on the same
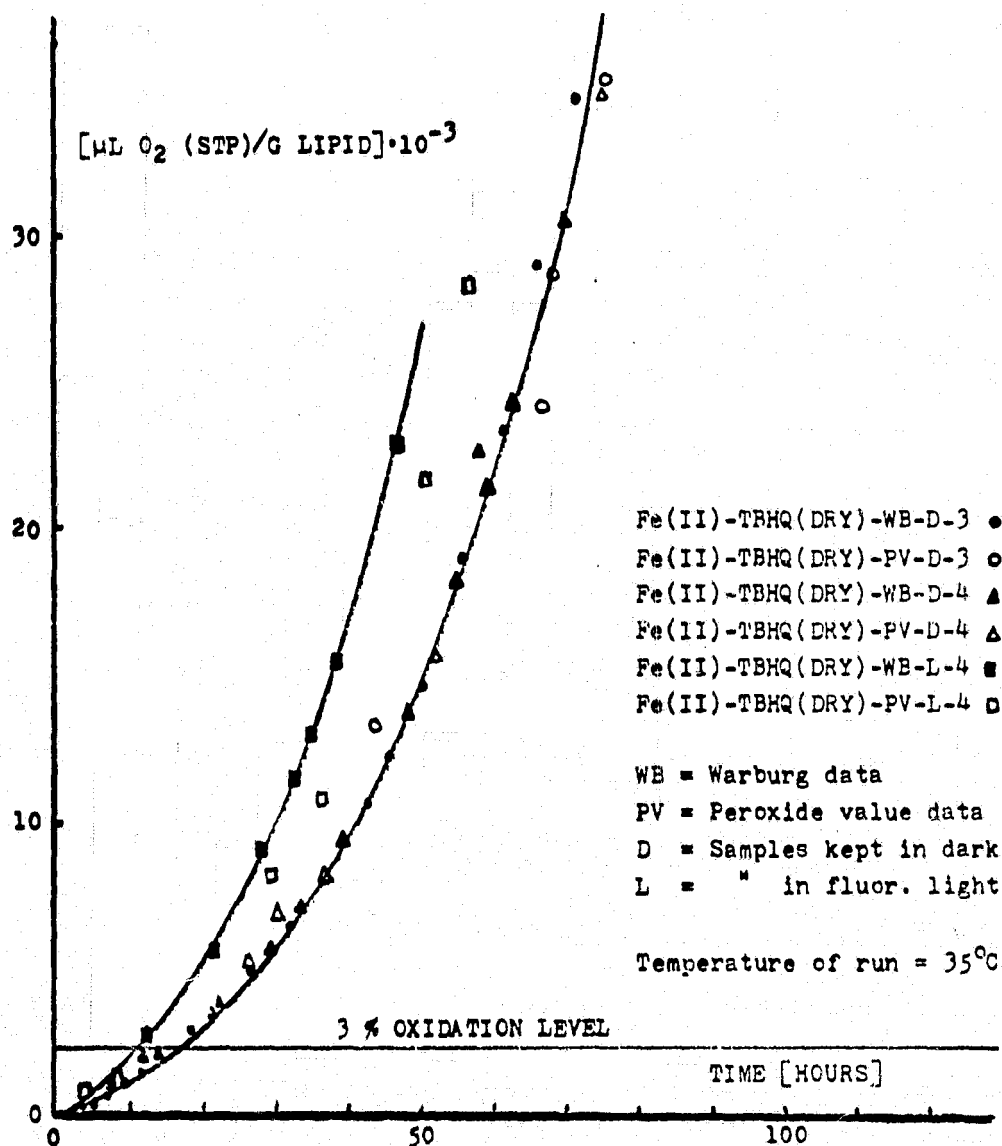
$[\mu L\ O_2\ (STP)/G\ LIPID] \cdot 10^{-3}$

Fe(II)-TBHQ(DRY)-WB-D-3 ●
Fe(II)-TBHQ(DRY)-PV-D-3 ○
Fe(II)-TBHQ(DRY)-WB-D-4 ▲
Fe(II)-TBHQ(DRY)-PV-D-4 △
Fe(II)-TBHQ(DRY)-WB-L-4 ■
Fe(II)-TBHQ(DRY)-PV-L-4 □

WB = Warburg data
PV = Peroxide value data
D = Samples kept in dark
L = " in fluor. light

Temperature of run = 35°C

3 % OXIDATION LEVEL

TIME [HOURS]

Figure 3   RESULTS FROM RUN 3,4 - WARBURG AND PV DATA

basis. As shown, there seems to be little hydroperoxide break-down even at very high levels of oxidation (40-50%). In . general in this study there was no evidence of significant hydroperoxide breakdown at the levels of oxidation measured (usually up to 15-20%). Only in the case of samples having very long induction periods (especially BHA and BHT at the lower temperatures) was there in some cases a significant lag between PV and O_2-uptake curves. The data indicate that this is due to the difficulty of maintaining the two systems under exactly the same conditions over extended periods of time. These results are somewhat surprising because the Fe(II) concentration is still quite high and iron is known to cause peroxide breakdown.

No correction factor for sample size was used in calculating peroxide values. Indeed no effect of sample size was observed on several occasions using this system indicating that results of PV method studies do not apply. Several tests were e.g. carried out in run 5 as to the effect of sample size on the PV measure-ments. No effect was seen for the low degrees of oxidation of interest.

In run 3 it was noticed that a sample kept in a VPM flask at room temperature had a much higher PV than an identical sample from an amber jar held at $35^{o}C$. It was suspected that light was a factor responsible for the acceleration. Figure 3 shows data from run 4 where the effect of light on the system containing TBHQ was investigated. The choice of TBHQ was

arbitrary. The results show that light indeed accelerated the rate decreasing the time needed to reach 3% oxidation from 15 to about 10 hr. Also for the first time, the oxygen uptake as measured by peroxide values did not quite match the actual oxygen uptake. It was speculated that the light was a factor in decomposing the hydroperoxides and thus speeding up the oxidation. It should be pointed out, however, that in this run the Warburg samples and the PV samples are not quite comparable. In the first case the light must go through the desiccator as well as a rather thick-walled glass (transparent) jar. In the latter case through thin-walled Warburg flask only.

Next it was decided to run a short experiment in order to test the effect of fluorescent light on the Fe(II) control, both dry and IM. The results are shown in Figure 4. Unfortunately as the run was started it was found that the PV of a brand-new batch of pure methyl linoleate to be used for the run was equivalent to 1500 μL O_2/G LIPID!

Despite this drawback, the data in Figure 4 show quite clearly that light speeds up the rate of lipid oxidation. The shortening of the induction period of the dry control was almost exactly the same (on a relative basis) as that observed for TBHQ (Figure 3). The effect on the IM system was considerably greater, however. Note also that coincidentally the rates of oxidation of the dry and IM controls ("dark") are similar. This phenomenon was also seen for Co(II) (Figure 1).
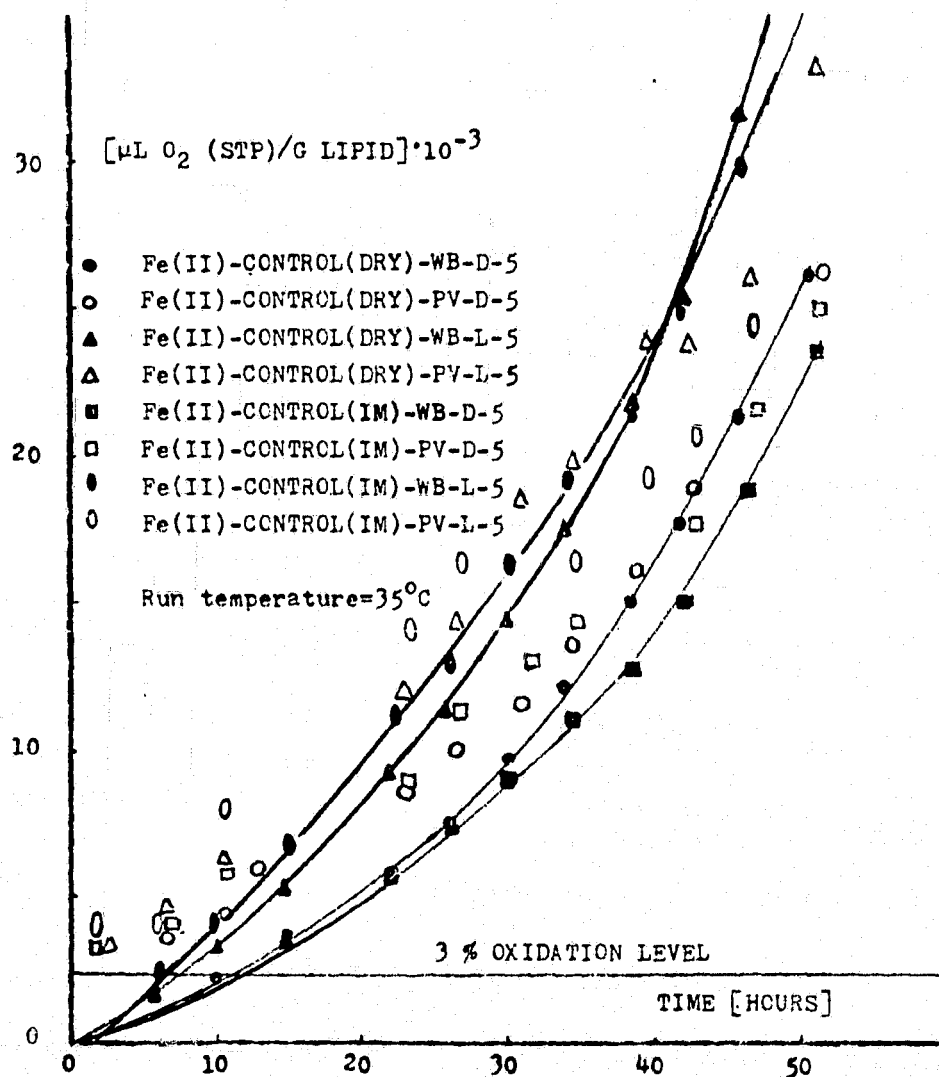
Figure 4   RESULTS FROM RUN 5 - WARBURG AND PV DATA

The peroxide value data should be treated with caution. But it seems that some breakdown is occurring in all the samples, especially for the "illuminated" samples. The reason for the scatter, especially in the "illuminated" samples in the PV-data is that in order to make the PV-samples and the Warburg samples strictly comparable, the former were kept in a number of Warburg flasks.

Since it is well known that water activity affects the rate of lipid oxidation the effect of 'small' a_w-changes was tested using a tocopherol system. In this case two different initial water activities were used, 0.096 and 0.24. It was found that the higher water activity considerably increased the induction period of the system with respect to lipid oxidation. The time needed to reach 3% oxidation (at $35^{\circ}C$) rose from 58 to 72 hr. This effect is in line with the theory that the lipid oxidation rate is at minimum close to the monolayer moisture content (the monolayer water activity for a glycerol-model system occurs at 0.25).

Figure 5 shows data taken from runs 9-11 at $45^{\circ}C$ using an IM system. The times needed to reach 3% level of oxidation were 8, 11 and 60 hours for control, EDTA and BHA respectively. The data show well the relative effectiveness of BHA, which in this case, increased the induction period over 7 times (PF = 7.25). The relatively poor performance of EDTA is somewhat disappointing. One might have expected greater efficiency in a model system where the EDTA is
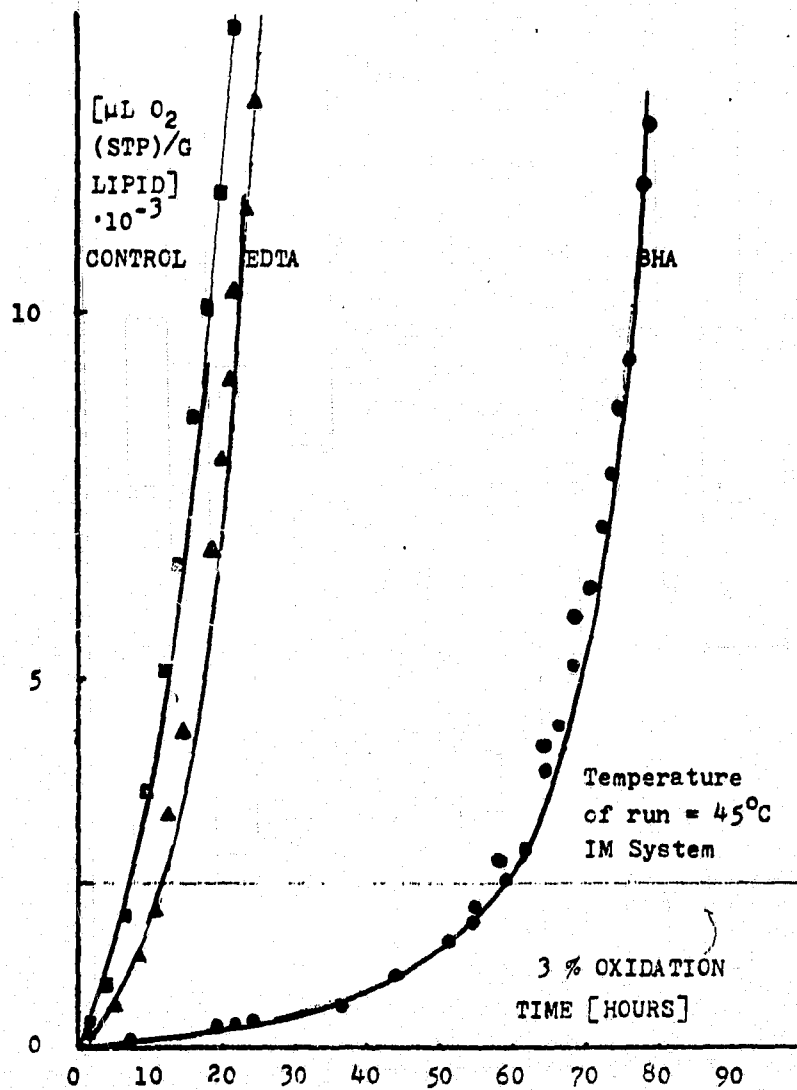
Figure 5   RESULTS FROM RUNS 9-11 - WARBURG DATA

relatively free to move about.

Table 9 is a compilation of data obtained in runs 2-11. The times needed for systems to reach 1% and 3% oxidation are included. So far model system studies at 25, 35 and 45°C and 35 and 45°C have been done at a_w 0.75 and 0.11 respectively.

The IM systems will be considered first. The order of effectiveness of the antioxidants in the IM system is (in order of decreasing protection): BHA, BHT, EDTA, E, TBHQ, IPC, PG, AA. Only BHT and especially BHA were seen to be good antioxidants, under all conditions used. EDTA and vitamin E were less effective giving 1.5-4 fold protection. TBHQ and IPC were almost totally ineffective whereas AA and PG were actually prooxidants under the conditions used.

The dry system data show a similar relationship. BHA and BHT are now more closely comparable. Again IPC and TBHQ were of no use and will not be considered further. Again the ascorbic acid is a prooxidant whereas PG now functions as a fairly potent antioxidant. The reasons for this behavior are not clear yet. In this system α-tocopherol was much better than EDTA.

In general it is obvious that of the antioxidants studied, the best one by far is BHA. BHT, especially in the dry system shows comparable, although clearly less, effectiveness. α-tocopherol is more effective in the dry system whereas EDTA appears to be more effective in the IM system. Only by doing a run at 25°C using the dry system will one know whether EDTA really is more effective at the higher water activities.

Table 9

TIME NEEDED TO REACH 1 AND 3% OXIDATION

IM system

$T^o C$	RATES	ANTIOXIDANTS								
		Control	IPC	TBHQ	AA	PG	E	EDTA	BHT	BHA
26	t_1	10			3.7	8.3	31.5	34	100	380
26	t_3	22.5			10.3	14.5	85	55	250	500
35	t_1	5.7	7.3	8.5		3.4	10		31	74
35	t_3	12.5	15.5	17.2		6.5	24		75	125
45	t_1	3.6			1.7	2.1	6.3	6.0	16	40
45	t_3	8.0			3.9	3.8	15.7	10.7	37	58

Dry System

$T^o C$	RATES	Control	IPC	TBHQ	AA	PG	E	EDTA	BHT	BHA
35	t_1	6.0	6	7		15	45		200	285
35	t_3	13.5	14	15.5		28	58		290	325
45	t_1	4.2			2.1	13.2	20.5	6.2	95	135
45	t_3	8.6			5.0	20.0	31.8	11.7	130	175

t_1 = time needed for sample to reach 1 % level of oxidation

t_3 = time needed to reach 3 % level of oxidation

Table 10

PROTECTION FACTORS

IM system

$T^{o}C$	PF's	Control	IPC	TBHQ	AA	PG	E	EDTA	BHT	BHA
26	PF_1	1			0.37	0.83	3.2	3.4	10	38
26	PF_3	1			0.46	0.64	3.8	2.4	11	22
35	PF_1	1	1.3	1.5		0.60	1.8		5.4	13
35	PF_3	1	1.2	1.4		0.52	1.9		6	10
45	PF_1	1			0.47	0.58	1.8	1.7	4.4	11
45	PF_3	1			0.49	0.48	2.0	1.3	4.6	7.3

Dry System

$T^{o}C$	PF's	Control	IPC	TBHQ	AA	PG	E	EDTA	BHT	BHA
35	PF_1	1	1.0	1.2		2.5	7.5		33	48
35	PF_3	1	1.0	1.1		2.1	4.3		22	24
45	PF_1	1			0.50	3.1	4.9	1.5	23	32
45	PF_3	1			0.58	2.3	3.7	1.4	15	20

PF = Protection factor

PF_1 = t_1 for antioxidant / t_1 for control

PF_3 = t_3 for antioxidant / t_3 for control

For definitions of t_1 and t_3 see table 9

Table 10 indicates the protection factors calculated on the basis of the data in table 9 simply be dividing the time to reach 1 % or 3 % oxidation for the antioxidant in question by time needed for the control to reach same level of oxidation. The data indicate very clearly that the protection offered by the antioxidants decreases with increased temperature. This seems to indicate that the antioxidants do have some effect on the activation energies. This finding is of considerable significance and indicates that one should be very careful to use very high temperatures in accelerated storage life tests since the relative protection offered by the antioixidants at this high temperature will not reflect actual protection at normal storage temperatures. The data for BHA are particularly striking. Here the protection factor dropped to less than half its original value when the temperature was increased from 26 to 35°C! The subsequent change from 35 to 45 was considerably less, however, indicating that the activation energy may not be constant when BHA is used. It should be emphasized that only by carrying out the correct kinetic analysis both on the control and the antioxidant systems can the effect of antioxidant and temperature be accurately evaluated.

D.   Summary and Conclusions

In conclusion, it has been shown that the relative effectiveness of antioxidants depends strongly on the water activity in many cases as is seen e.g. with vitamin E. In one case, for propyl gallate, the antioxidant became a prooxidant in the IM range. Secondly, it has been shown that

the effectiveness depends strongly on temperature. In general there is a greater drop in effectiveness in going from 26 to $35^{\circ}C$ than there is between 35 and $45^{\circ}C$. Thirdly it has been shown that in presence of 50 ppm Fe(II) (lipid basis) only BHA and BHT behave as strong antioxidants. In general the phenolic antioxidants were more effective in the dry system. Unlike the data obtained with a Co(II)-system from previous studies in this department EDTA was not very effective. The fat soluble analogue of citric acid, isopropyl citrate was completely useless and so was the new antioxidant recently permitted by the FDA, tertiary butyl-hydroquinone or TBHQ. Finally ascorbic acid approximately doubled the rate of oxidation.

Page intentionally left blank

VII. Study of Water Activity of Cheese and Preparation of an Intermediate Moisture Process Cheese

A. Introduction

If properly manufactured and packaged, most cheeses can have a relatively long shelf-life at refrigerated temperature. This is mainly due to the heat treatment given the milk and the acids developed during fermentation of the cheese. However, microbial spoilage, especially by molds, is a rather common problem. Under certain conditions, food pathogens may even grow in cheese.

During the fall of 1965, there was an outbreak of staphylococcal food poisoning involving cheddar, Monterey and Kuminost cheeses in the U.S. (Zehren and Zehren, 1968). In early 1974, Argentina confirmed a botulism outbreak caused by commercial cheese spread (personal communication).

These public health problems suggest that the water activity in cheese is rather high. Since water activity (a_w) has an important effect on the control of growth of microorganisms (Scott, 1957; Troller, 1973), information on the a_w of cheeses would be valuable in assessing microbial stability and potential for food poisoning. Unfortunately, no such information is available in the literature. One of the objectives of this study was to determine the a_w and pH of various kinds of commercial cheeses so as to assess their stability characteristics. In addition to this it was decided to attempt to make an intermediate moisture process cheese which would be shelf stable at room temperature with absolute assurance against microbial growth.

Process cheese is made by grinding, mixing and heating one or more kinds of natural cheeses together with emulsifying salts, water and coloring agents. The whole mass is worked into a homogeneous, plastic mixture. The final product sets to a semisolid form upon cooling (Thomas and Hyde, 1972; Thomas, 1973). Process cheese was first manufactured in Switzerland in 1911. The initial purpose was to re-process cheese with textural defects and to provide a product suitable for tropical countries with poor refrigeration facilities. The main advantages of process cheese over the natural cheese were better keeping quality and greater uniformity.

The processing steps are outlined as follows:

1. Selection and blending of natural cheeses

2. Cleaning and trimming

3. Milling

4. Incorporation of emulsifiers, water, salt and color

5. Cooking

6. Packaging and storage

For blending, selected natural cheeses are mixed to obtain desirable physical characteristics, pH and chemical composition in the product. After cleaning and trimming, the cheese is weighed, cut into pieces, and then fed into a grinder. The milling enables the emulsifying salt to come into good contact with the cheese and helps in the melting process.

Emulsifying salt plays an important role in the process by preventing the separation of fat and moisture. It ties up the calcium ions of the protein and changes it from an insoluble form (paracasein)

to a more soluble form (casein). The soluble protein binds water and forms a protective film around the fat globules, thus stabilizing the emulsion. Citrates and phosphates are common emulsifying salts.

The quality of processed cheese depends on the pH. For optimum results, the pH should fall between 5.4 and 5.7. If the pH falls too low, the cheese tends to be crumbly or short-bodied. If the pH is above 6, the flavor might become bitter and fat may separate out. Also there is a danger of deterioration.

Heat and agitation are required to convert the mixture into a smooth, homogeneous mass. Processing also stabilizes the product by inactivating the enzymes and destroying the vegetative microorganisms present (including most pathogens) since temperature used is about 175 to 185°F for 6 to 12 min. The common cooking equipment used is a steam-jacketed kettle equipped with direct steam injection and agitator.

Processed cheese is packaged hot from the cooker in aluminum foil, plastic film-lined cans or boxes. Rapidly cooled processed cheese is usually soft, a slower cooling produces a firmer body. The product can be stored at normal room temperature without spoilage. A good quality process cheese should possess good flavor, be easy to slice, not crumble easily and be reasonably firm and elastic.

Although process cheese can usually be stored for a prolonged period with no refrigeration, it is not sterile and heat resistant spores could germinate under favorable conditions. Also contamination by bacteria and molds can occur if the wrapper is damaged or not properly sealed, especially after opening. As stated, the other objective of

this study was to produce a shelf stable process cheese in the intermediate moisture range by the addition of milk solids, humectants and antimycotic agents (Bone, 1969; Brockman, 1970). The product should retain the basic characteristics of regular process cheese and withstand adverse storage conditions such as high ambient temperature and faulty packaging. No attempt was made to conform to the federal regulations of process cheese, however, only FDA-approved ingredients and additives were used.

B. Materials and Methods

    1. A_w measurement of commercial cheese

        Thirty assorted cheeses were purchased from a local store. The a_w of each sample was determined at room temperature (23°C) by the Fett-Vos method (Fett, 1974; Vos and Labuza, 1974) and the vapor pressure manometer (VPM) technique (Labuza, 1974). The pH of all cheese samples except Parmesan was determined by direct measurement (Hausler, 1972) using a Beckman non-aqueous electrode No. 39142 together with a Copenhagen radiometer (type PHM276). The dilution method (Thomas and Hyde, 1972) was used for the Parmesan cheese.

    2. Preparation of intermediate moisture process cheese

        Nine-month old cheddar cheese was obtained from Land'O'Lakes (Minneapolis, MN). The cheese was weighed, cut into pieces and then milled with a Hobart meat grinder. Four different batches were prepared and the compositions are shown in Table 1.

        A Damrow (Fond du lac, Wis) steam cooker (pilot plant model) equipped with agitator, steam jacket and direct steam injection was used for processing. The cooker was preheated to 120°F and the cheese added with agitation. Other ingredients were then blended into

TABLE 1

COMPOSITION (lb) OF INTERMEDIATE MOISTURE PROCESS CHEESE

Ingredient	System			
	No. 1	No. 2	No. 3	No. 4
Cheddar cheese	30	30	30	30
Non-fat dry milk	10	10	10	11
Disodium phosphate	1	1	1	1
Propylene glycol	—	2	1	1.5
Butter	—	1	0.5	0.5
Salt	—	0.5	1	1
Sodium citrate	—	0.1	0.1	0.2
Potassium sorbate	—	0.044	0.044	0.044

the cheese. The mixture was heated to 180°F and the product was
removed from the cooker. The total processing time was approximately
20 min. The cooked cheese was filled into 2 lb. boxes with a cellophane
liner. The products were then cooled to 4°C.

Sensory evaluation was conducted on the four systems
using a 9-point hedonic scale. The panel consisted of 10 members of
the Food Science Department. The samples were tested for flavor,
off-flavor, texture, appearance, aroma and overall acceptance. A_w
and pH were determined using the VPM method and the direct measurement
method, respectively.

C. Results and Discussion

Table 2 shows the a_w and pH values of the 30 commercial
cheeses. With the exception of Parmesan and whey cheese, all the
samples tested showed high a_w (>0.94). Many cheeses had an a_w very
close to 1.0. The a_w values determined by the two different techniques
do not differ more than 0.2 units in most cases. Labuza et al.
(1975) compared several different methods in a_w determination and found
that the data for high a_w samples were quite variable. It is difficult
to obtain good accuracy at high a_w range.

For a few samples a_w measurement using the VPM technique was
not possible since an equilibrium condition could not be attained.
The gradual, continuous increase of the manometer reading was due to
the respiration of microorganisms or release of volatiles from these
cheeses(Labuza et al., 1975). According to the results in Table 2,
most cheeses show very high a_w and therefore are subject to microbial
spoilage and growth of S. aureus especially if not held at refrigerated

TABLE 2

WATER ACTIVITY AND pH OF 30 COMMERCIAL CHEESES

Cheese	Manufacturer	Fett-Vos	VPM	pH
Cracker barrel (cheddar)	Kraft	0.97	0.98	5.3
Canadian cheddar	Purity	0.95	0.95	5.2
Longhorn colby	Byerly's	0.99	0.98	5.3
Bongard's colby	Bongard's	0.99	1.0	5.0
Monterey Jack	Purity	0.99	1.0	5.8
Mozzarella	Kraft	1.0	1.0	5.8
Parmesan (grated)	Kraft	0.76	0.75	5.4
Romano	Kraft	0.97	1.0	5.3
Provolone	Kraft	0.98	1.0	5.4
Swiss	Kraft	1.0	0.97	5.7
Gouda	Purity	0.99	1.0	4.7
Edam	Purity	1.0	——	5.3
Muenster	Purity	1.0	0.99	5.4
Farmer cheese	Purity	0.99	0.96	5.8
May Bella	Purity	0.98	——	5.0
Jackie	Denmark	0.99	——	5.4
Whey	Sweden	0.91	0.88	5.8
American (process cheese)	Kraft	0.98	0.97	5.8
Hoffman's process cheese	Anderson Clayton	0.97	0.97	5.8
Pot (processed cottage)	Milwaukee	1.0	1.0	5.1
Fondue Swiss Knight	Switzerland	1.0	——	5.5
Kaukuna Klub (cold pack cheddar)		0.96	1.0	4.8

Table 2 cont'd

Ye Old Tavern (cheddar)		0.96	0.95	5.1
Swiss-American spread	Kraft	0.96	0.93	5.6
Velveeta (spread)	Kraft	0.96	0.99	5.8
Brie	Fromagerie Bongrain Inc	1.0	1.0	7.4
Camembert	France	1.0	—	6.1
Camembert	Denmark	0.99	—	7.0
Blue	Treasure Cave	0.94	—	5.1
Blue Stilton	England	0.94	—	5.8

temperature. The inhibitory effect of the acids in cheese is mainly responsible for retarding the growth of any microorganism present if held at refrigerated temperature.

The pH of the cheeses ranged from 4.7 to 5.8 in most cases. Camembert and Brie cheeses showed exceptionally high pH. This is probably due to the ripening by the molds with degradation of proteins.

The taste panel results for the four process cheese systems prepared in the lab are summarized in Table 3. The first sample, which was obtained by blending 30 lb. of cheese and 10 lb. of non-fat dry milk (NFDM) (Table 1), turned out to be hard and dry. The inferior texture was reflected by the low texture score of 4.2 (dislike). Furthermore, some of the NFDM solids did not dissolve during cooking and formed clumps in the cheese, rendering it undesirable.

Different levels of propylene glycol, together with salt and antimycotic agents, were incorporated into the other three systems (Table 1). Besides acting as an effective water binder (Brockman, 1970), propylene glycol also helps to solubilize the milk solids, increase the plasticity of the product and prevent microbial growth. It was noted that the NFDM solids completely dissolved in these samples during the cooking. Also, the texture of the product was improved significantly by adding the glycol (Table 3).

Samples No. 2 and 3 rated substantially better than the other two, with overall acceptance scores of 7 (like). Considerable browning occurred in system No. 4. This is probably caused by the increase in milk solid content which is high in lactose. The cheese also tended to crumble and had poor sliceability.

TABLE 3

SENSORY EVALUATION OF FOUR INTERMEDIATE MOISTURE PROCESS CHEESES

System	Flavor	Off-flavor	Texture	Appearance	Aroma	Overall	Comments
1	6.5	5.8	4.2	6.2	6.8	5.5	Hard, dry
2	6.4	6.2	6.9	7.2	7.8	7.1	Off-flavor, sweet
3	7.2	5.6	6.5	7.6	7.5	7.0	Sweet, salty
4	6.4	4.7	6.6	5.9	7.0	5.9	Dark, salty off-flavor

The off-flavor from propylene glycol was quite noticeable when the cheese was hot. However, as the cheese cooled down, the off-flavor became hardly discernable. Most panelists were able to pick up the more intense salty flavor in samples No. 2 and 3. However, this did not have much effect on the flavor scores. The salt was added as a preservative and water binder. Judging from the taste panel results, systems No. 2 and 3 appear to be quite satisfactory and therefore should have market potential and usefulness in the space shuttle system.

The a_w and pH values of the four cheese systems are shown in Table 4. The first sample had an a_w of 0.85. The incorporation of propylene glycol and salt to the cheese reduced the a_w by 0.02 and 0.03 units, as shown in the other samples. The pH of the last two systems was below the optimum range, 5.4 to 5.7 (Morris, 1972; 1973). However, this is desirable from an antimicrobial standpoint. No storage study was conducted on the products but they were examined after two weeks of storage at 40^OF. No fat separation, browning or significant change in texture or flavor occurred, however, some lactose may have crystallized on the surface. This should not occur at room temperature. According to Acott and Labuza (1975) and Boylan et al. (1975), the combination of propylene glycol, potassium sorbate and sodium citrate should inhibit the growth of Staphylococcus aureus and molds at room temperature in IM foods, especially a. a_w 0.8? to 0.83. It is envisioned that systems No. 2 and 3 should be shelf stable to all microorganisms. Also, Warmbier et al. (1975) indicated that propylene glycol may help to retard non-enzymatic browning in the cheese. Further studies on

TABLE 4

WATER ACTIVITY AND pH OF FOUR

INTERMEDIATE MOISTURE PROCESS CHEESES

Sample No.	a_w	pH
1	0.85	5.4
2	0.83	5.4
3	0.82	5.2
4	0.82	5.2

the microbiological and chemical stabilities of the products would be necessary to ensure a long shelf life.

In conclusion, this study indicated that most commercial cheeses have a_w's above 0.95 and therefore are subject to spoilage. Also, it has been shown that process cheese with an a_w of 0.83 can be prepared by the addition of NFDM solids, salt and propylene glycol. Two of the systems prepared were placed in the "like" category (mean score = 7) in overall acceptance based on a nine-point hedonic scale. These products are expected to be shelf stable at room temperature even under adverse storage conditions.

D.   References

Acott, K. and T.P. Labuza. 1975. Inhibition of Aspergillus niger in an intermediate moisture food system. J. Food Sci. 40: 137.

Bone, D. 1969. Water activity - Its chemistry and applications. Food Product Dev. 3(5): 81.

Boylan, S., K. Acott and T.P. Labuza. 1975. Staphylococcus aureus F265 challenge studies in an intermediate moisture food. J. Food Sci. (submitted).

Brockman, M.C. 1970. Development of intermediate moisture foods for military use. Food Technol. 24: 896.

Fett, H.M. 1974. Water activity determination in foods in the range 0.80 to 0.99. J. Food Sci. 38: 1097.

Hausler, W.J., Jr., editor. 1972. Standard Methods for the Examination of Dairy Products. 13th Edition. American Public Health Assoc., Inc. Washington, D.C.

Labuza, T.P. 1974. Theory, Determination and Control of Physical Properties of Food Materials. D. Reidel Publishing Co., Dordrecht, Holland. Chapter 10, pg. 119.

Labuza, T.P., S.R. Tatini, K. Acott, R. Lee, J. Flink and W. McCall. 1975. Determination of water activity: A comparative study. J. Food Sci. (submitted).

Scott, W.J.   1957.   Water relations of food spoilage microorganisms.
    In Advances in Food Research.   Mrak, E.M. and G.F. Stewart, editors.
    Vol. 7, pg. 83.   Academic Press, New York.

Thomas, M.A. and K.A. Hyde.   1972.   The Manufacture of Processed Cheese –
    A Working Manual.   Bulletin. D40, Div. of Dairying, New South
    Wales Dept. of Agriculture.

Thomas, M.A.   1973.   The Manufacture of Processed Cheese – Scientific
    Principles.   Science Bulletin 84, New South Wales Dept. Agriculture.

Troller, J.A.   1973.   The water relations of food-borne pathogens.
    J. Milk Food Technol.   36: 276.

Vos, P.T. and T.P. Labuza.   1974.   Technique for measurement of water
    activity in the high a_w range.   J. Agric. Food Chem.   22: 326.

Warmbier, H.C., R. Schnickels and T.P. Labuza.   1975.   Effect of
    glycerol on non-enzymatic browning in a solid intermediate
    moisture model food system.   J. Food Sci.   (submitted).

Zehren, V.L. and V.F. Zehren.   1968.   Relation of acid development
    during cheese making to development of staphylococcus enterotoxin
    A.   J. Dairy Sci.   51: 645.

VIII. Acknowledgements

The following contract and grants also contributed in part to the total project and should be acknowledged.

·University of Minnesota Agricultural Experiment Station

Projects 18-52 and 18-72

·Quaker Oats Company Intermediate Moisture Food Research Fund